THE JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

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THE JOURNAL OF EXPERIMENTAL MEDICINE.

The support which the medical profession has given to THE JOURNAL OF EXPERIMENTAL MEDICINE during the first year of its existence has been most gratifying to those interested in the establishment of an American journal devoted to the publication of original contributions to the medical sciences. With the continuance and natural growth of this support the future of the Journal will be assured.

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THE EDITOR.

January, 1897.

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ON THE PRESENCE OF NERVES IN TUMORS AND OF OTHER STRUCTURES IN THEM AS REVEALED BY A MODIFICATION OF EHRLICH'S METHOD OF "VITAL STAINING" WITH METHYLENE BLUE.

BY HUGH H. YOUNG, M. D.
(*From the Anatomical Laboratory of the Johns Hopkins University.*)

PLATE I.

Our knowledge of neoplasms, notwithstanding the enormous amount of research to which they have been subjected, is still extremely vague and unsatisfactory. With nearly every advance in histological technique, however, its application to tumors has afforded some additional fact or facts concerning them, and it is only by utilizing for their study the various improvements of older methods or entirely novel procedures, that we may hope for the accumulation of data from which may be deduced a satisfactory explanation of their origin and somatic relations. About a year ago, at the suggestion of Dr. Lewellys F. Barker of the Anatomical Laboratory, I undertook the study of some of the tumors obtainable in the surgical operating room of the Johns Hopkins Hospital, with Golgi's silver-method and with a modification of the method of vital staining by methylene-blue introduced by Ehrlich* of Berlin. I have to thank Dr. Barker for instruction in the use of

*Ehrlich, P., Ueber die Methylenblaureaction der lebenden Nervensubstanz. *Deutsche med. Wochenschr.*, 1886, No. 4.

the methods and for the interest he has manifested in the progress of the research. The silver-method, after repeated trials even with double and triple impregnation, was found to afford such uninteresting results with these tissues that it was discarded. The staining with methylene-blue, however, yielded many striking pictures, and the method as modified may be warmly recommended for revealing certain of the details in fresh pathological tissues as obtained from the living patient. The present studies pretend to no degree of completeness, but since they include a number of observations which would seem to be entirely new, and because for unavoidable reasons the research must for the present be discontinued, I have decided to put the results on record, with the hope that others may be sufficiently interested in them to make further experiments along similar lines.

METHODS AND MATERIAL.

Naturally with human beings, although small doses of the substance appear not to be injurious, the injection *intra vitam* of amounts of methylene-blue sufficient to stain the nerves, as practised by Ehrlich in animals, is out of the question. It has been shown, however, by those who have worked extensively with the methylene-blue staining (Arnstein, Dogiel, Mayer, Smirnow, Apathy and others) that immersion of small pieces of tissue, removed from the animal while living or immediately after death, in dilute solutions of methylene-blue affords results almost, if not equally, as good as those obtained by injection. Accordingly, in my work thin layers of tissue cut with the double knife of Valentine were employed altogether. After acquiring experience in the use of the stain on embryonic tissues in which the nerve fibres and nerve endings are very easily demonstrable, the examination of tissues from adult human beings and of those from the interior of solid tumors was undertaken. Solutions of methylene-blue* of varying strength and consistence were tried with variable results. The suggestions of Lavdowsky† with regard to the use of

* Grübler's "Methylen-blau rectif. nach Ehrlich."

† Lavdowsky, M., Zur Methodik der Methylenblaufärbung und über einige neue Erscheinungen des Chemotropismus. *Ztsch. f. wiss. Mikr.*, Bd. xii (1895), Heft 2, p. 177.

egg albumin in the mixtures were found particularly serviceable. After many trials, the following proportions of materials were selected as affording the most constant results:

Fresh agg albumin,
Physiological salt solution,
0.25 per cent aq. sol. ammon. chlorid.,
1 per cent aq. sol. methylene-blue; of each 10 cc.

These constituents after thorough mixing make a stain ready for immediate use. It must be freshly prepared for each experiment. The sections cut from the tumor are placed upon large glass slides and surrounded on all sides with the stain. The slides are then placed in a warm, moist chamber and examined at intervals under a low power of the microscope. In from 45 minutes to 2 hours, when the acme of differential staining has been reached, the tissues are plunged into the ice-cold fixing fluid of Bethe,* which has the following formula:

Ammon. molybdat.,	1 gramme.
Distilled water,	10 cc.
Hydrogen peroxide,	1 cc.
Acid. hydrochlor.,	1 drop.

The bottle containing the fixing fluid and the specimens is kept in the ice-box over night, after which the bits of tissue are washed carefully in running water for from one to two hours, then quickly dehydrated in cold absolute alcohol, cleared in xylol and embedded as soon as possible in paraffin. Serial sections are then cut and fastened to the slide with Mayer's albumin or by the alcohol method; they may be mounted without further staining in xylol balsam, or if desired—and this has been in many instances of distinct advantage—a differential stain of alum cochineal may be employed after removal of the paraffin. The sections may then be studied, best of all with oil-immersion lenses. The procedure as above outlined, though far more reliable than the early methylene-blue methods recommended, affords by no

* Bethe, A., Angaben über ein neues Verfahren der Methylenblaufixation. *Archiv f. mikr. Anat.*, 1895, Bd. xliv.

means constant results. After some experience with it, however, microscopic pictures, often of very striking beauty, are obtainable

The material used in the investigation, with the exception of the epithelioma of the uterus, which came from the gynæcological service, was drawn entirely from the patients in Prof. Halsted's operating room. I wish to thank Drs. Halsted, Finney and Bloodgood for kindly supplying me with the tumor masses immediately after their excision. Up to the present time I have studied the following growths by this method:

1. Scirrhous carcinoma of the breast.
2. Epithelioma of cervix uteri.
3. Scirrhous carcinoma of breast.
4. Subperiosteal sarcoma of tibia.
5. Scirrhous carcinoma of breast.
6. Myxoma of axilla (very painful).
7. Metastatic carcinoma of glands of neck.
8. Sarcoma of muscles of leg.
9. Mass of mycosis fungoides.
10. Subperiosteal sarcoma of condyle of femur.
11. Intracanalicular myxoma of breast.
12. Amputation neuroma (right arm).
13. Myxoma.

THE PRESENCE OF NERVES IN TUMORS.

The question of the relation of nerves to tumors, aside from true neuromata and tumors of nerves, is practically *terra incognita*. A careful search through the bibliographic references in the catalogue of the Surgeon-General's Library and in the Index Medicus shows no direct reference to the subject. The general text-books on pathology are either entirely silent regarding this topic or dismiss it with an acknowledgment of our ignorance. Thus in Green* we are told that tumors contain no nerves. Senn† says: "But little is known of

* Green, Pathology and Morbid Anatomy, 1889.

† Senn, N., Pathology and Surgical Treatment of Tumors, 1895.

the innervation of tumors. The tenderness and spontaneous pain which belong to certain varieties of tumors would suggest the presence of new nerve fibres and should induce pathologists to make additional researches." No mention is made of nerves in tumors either in the last edition of Ziegler's Pathology* or in Thoma's text-book on the same subject.† The following quotation from Williams well illustrates the poverty of results as yet obtained in this field of investigation: ‡ "Cancers are regarded as nerveless, but vaso-motor filaments probably accompany the stromal blood-vessels, although this is denied by Verneuil and Nefvers. Our knowledge is singularly inadequate."

In their recent work on tumors, Luecke and Zahn§ state that concerning the innervation of tumors but little is known, although from a clinical standpoint no doubt of the presence in them of nerves can be entertained. Thus the possibility of quieting the pain in tumors by injection into them of anodynes speaks for the presence of nerves. It is probable, however, in their opinion, that the nerves were present before the formation of the tumor, and that none are newly formed. They refer to a report of a case by Billroth of nasal polyp, in which he found nerve fibres which he considered to be newly formed. But in a similar tumor examined by Zahn, which likewise contained nerve fibres near its origin, these were thought by him to be without doubt old preformed fibres.

In view of the foregoing statements, the findings in the cases included in the present study can scarcely fail to be of general interest; excluding tumor No. 6, which was very painful and probably represents a myxoma developing in the course of a small cutaneous nerve, the mycosis fungoides (No. 9) and the amputation neuroma (No. 12), we have left ten definite new growths which represent neither tumors developing from nerves nor true neuromata. In no less than five of the ten the presence of nerves was positively demonstrated, and in

* Ziegler, E., Lehrbuch der allgem. u. spec. path. Anat., 1896.

† Thoma, R., Text-book of General Pathology and Pathol. Anatomy. Trans. by Bruce, London, 1896.

‡ Diseases of the Breast, 1894.

§ Chirurgie der Geschwülste, Teil 1, p. 18, 1896.

some of them nerve fibres were present in considerable numbers, even in the minute pieces submitted to examination. Thus in No. 1, a carcinoma of the breast, a bundle of medullated nerves is visible adjacent to a milk duct, both being closely surrounded by carcinomatous tissue. In tumor No. 2, an epithelioma of the cervix, while the progress of the staining was being watched under a low power, a distinct though small bundle of nerve fibres containing four axones took the stain intensely and could be followed directly into the carcinomatous tissue. In tumor No. 3, a carcinoma of the breast in which the skin of the nipple is almost entirely replaced by cancerous tissue, a large bundle of medullated nerves has taken the stain. The possibility, however, that this may be a cutaneous nerve which has been surrounded by the ingrowing neoplasm cannot be excluded.

In tumor No. 4, a small round-celled sarcoma of the tibia, a small bundle of non-medullated nerves is to be seen closely bound to a small artery and vein by a moderate sheath of connective tissue. The section in this instance was taken from the middle of the tumor, and these vessels and nerves are near the centre of it and entirely surrounded by sarcoma cells. (Plate I, Fig. 1.)

In No. 10, a subperiosteal sarcoma of the lower end of the femur containing both spindle cells and round cells, three different bundles of nerves were made out in different sections. The tumor was very soft and showed large areas of œdema. The largest bundle of nerves —composed of about 50 medullated fibres—is accompanied by an artery and two veins, and is surrounded by a zone of œdematous fibrous tissue around which are closely packed sarcoma cells. Another bundle of six or more small axis cylinders accompanied by a vein and artery is surrounded by sarcomatous tissue, which is œdematous for a short distance and then becomes denser. One of these axis cylinders can be seen dividing, the branch accompanying the main fibre for a short distance and then becoming lost to view. The third and smallest bundle, composed of about five very fine axones, could be followed in its course among densely packed sarcoma cells. It was situated in the neighborhood of two minute blood-vessels belonging to the tumor.

The results even in these few cases would seem to justify the conclusion that nerves are of much more frequent occurrence within tumors than has previously been suspected. The examination of such minute particles of the tumors as those involved in these studies having revealed the presence in considerable numbers of nerves in one-half of the specimens looked at, it may certainly be assumed that, were it practicable to examine the whole of a tumor mass for its constituent nerves, (1) the number in some instances would be found to be enormous, and (2) the percentage of cases in which nerves are present would be even larger than this investigation would indicate.

More important, however, than the demonstration of the frequent occurrence of nerves in tumors is the question of the actual relation of the nerves to the tissue making up the tumor. Are these nerves so frequently present in the interior of tumors to be regarded simply as normal structures belonging to the tissues in which the tumor appears, which happen to have been included by the neoplastic tissues in their onward march, or are there grounds for believing that under some circumstances the nerve fibres belong to the tumor proper, forming really an integral part of it? While more extended research is essential for the establishment of the exact relations in all the different forms of tumors, I believe that my specimens justify the statement that in sarcomata, at least, there may be nerves which are as much an integral portion of the tumor substance as are the sarcomatous blood-vessels. The study of tumor No. 4 and of tumor No. 10, both subperiosteal sarcomata, shows the existence of axis cylinders within the central parts of the tumors in places in which they are not accompanied by tissues foreign to the tumors and where they are widely removed from the normal structures at the periphery. (Plate I, Fig. 1.)

As to the function of these nerves we can as yet only speculate. It is not impossible, inasmuch as in the two sarcomata mentioned they accompanied blood-vessels, that a part of them at least may be connected with the vaso-motor system. In this connection the distribution of the nerve terminals would be of help, but unfortunately these were not met with in the sections studied, and if they were present they remained unstained. In view of this deficiency further investigations will be required to clear up this point.

As to the nature of the nerves present in the carcinomata studied, I am unable to state positively that any one of them represents a portion of the tumor *per se*; indeed, for the present I rather favor the view that those found represent nerves of normal structures which have been surrounded by the invading cancer cells. It would not be surprising, however, if subsequent investigations should prove the presence, at least in the stroma of cancers, of nerves which may properly be considered actual parts of the new growth.

In No. 6 of the series, a painful tumor of the left axilla, when the capsule was cut, the tumor, which was smooth and glistening, was found attached to it only at one point by a narrow fibrous band in which no nerves could be definitely made out macroscopically. A microscopic section of the tumor showed, however, that it contained a large number of nerves. The axones stained brightly in the methylene-blue, and varied from large varicose fibres to very fine wavy fibrils. About some of the axones no medullary sheath could be detected. The individual fibres were separated from each other by varying amounts of myxomatous tissue and were not bound together by a common sheath. The total number of nerve fibres seen in this tumor is very great. This tumor must, therefore, be looked upon as a myxoma developing in the course of a small subcutaneous nerve and having its origin probably in the endo- or peri-neurium. The findings in this case account for the fact that clinically this tumor was extremely painful.

The amputation neuroma (No. 12) was studied simply as a matter of interest in order to observe the relations of the nerves to the scar tissue as revealed by the vital method of staining, and the results of course have no bearing upon the special subject here under consideration. Enormous numbers of multiply branched axones were easily demonstrable in the dense cicatricial tissues.

ON CERTAIN CELLS AND CELL INCLUSIONS IN TUMORS WHICH STAIN BY THIS METHOD.

a. Mastzellen and Plasma-cells.—The fact that with the vital staining method Mastzellen have a special affinity for the dye, a fact em-

phasized by Lavdowsky and others, I have frequently confirmed in studying sections of the skin and tongue, but I was surprised to find them in very considerable numbers in many of the tumors examined, both sarcomata and carcinomata. The forms assumed by the Mastzellen are various, but correspond closely to those met with in normal tissues. Two types predominate: (1) a form showing long processes, the protoplasm of the cell being filled with distinct granules which take a deep blue stain; and (2) a round or oval type smaller than the foregoing, the protoplasm tending to stain almost diffusely, though with the high powers extremely fine granules can be made out. Many of these probably correspond to what have been described as plasma cells. The Mastzellen, though most frequent about blood-vessels, may lie apparently anywhere among the tumor cells which do not take the stain.

A review of the bibliography teaches that Mastzellen have been before observed in various tumors by different investigators who used the older methods. Thus they are described by Hansemann* in the stroma of cancers, by Unna† in neuro-fibromata, and in leio-myomata by Jadassohn,‡ Gottschalk § and Reich.¶ In my methylene-blue preparations the Mastzellen form one of the most striking appearances in the microscopic picture, and the method affords perhaps the clearest demonstration of them in the tissues of tumors.

b. Red Blood Corpuscles.—In tumors as well as in other tissues some of the red blood corpuscles stain intensely with methylene-blue soon after its application. No pathological change has been made out in those which stain, and the proportion of red corpuscles taking the stain varies in different cases.

c. Cell Inclusions.—In tumor No. 4, a sarcoma of the tibia, numerous cells are to be seen which contain within their protoplasm certain irregularly shaped bodies or granules which take an intense blue stain.

* Hansemann, Das Krebsstroma und die Grawitz'sche Theorie der Schlummerzellen. Virchow's *Archiv*, Bd. cxxxiii, p. 147.

† Unna, Die Histopathologie der Hautkrankheiten, 1894.

‡ Virchow's *Archiv*, Bd. cxxi, 1890, p. 88.

§ *Arch. f. Gynäk.*, Bd. xliii, 1893, p. 534.

¶ *Arb. aus d. path. Inst. in Göttingen*, 1893, p. 216.

These cells, which differ entirely in appearance from Mastzellen, are generally larger than the other sarcoma cells adjacent. They appear swollen; their protoplasm is pale, but their nuclei are well preserved, showing no degenerative changes and staining well in alum cochineal.

The intracellular blue bodies vary in size from very minute granules, to be made out only with oil-immersion lenses, to bodies one-third the size of the nucleus of the cell. The smallest and some of the larger granules appear to be solid, but the majority of the larger bodies are vacuolated, showing an unstained or faintly stained centre surrounded by a deeply stained irregular granular peripheral ring. In shape they are ordinarily nearly spherical, but some of them are oval and some are even crescentic or horseshoe-shaped. Usually from three to five are found in a single cell, but as many as ten or more may occur. They lie in the protoplasm, have no apparent connection with the nucleus or with each other, being as a rule discrete, though sometimes they are massed together in heaps. Very different sizes and forms of the granules and bodies may be seen in one and the same cell. (Plate I, Fig. 2.) Similar solid extra-cellular bodies as well as vacuolated forms are to be seen lying apparently free among sarcoma cells, but they are not nearly so numerous as those within the cells.

In No. 10, another sarcoma of bone, similar bodies are to be seen, but they are smaller and not nearly so numerous as in the tumor just referred to. They are present in No. 8 (also a sarcoma), but in only a few of the cells. A careful search for them was made in the other tumors, but they were not found except in a carcinomatous skin nodule. Here in an alveolus of cancer cells some distance from the skin two cells containing similar vacuolated blue bodies are to be seen. None of the epidermal cells contain them.

We are ignorant as to the character of these bodies. They do not appear to be identical with Russell's fuchsin bodies,* which are believed by some to be confluent Mastzellen granules, by others to be proteid coagula,† since they are said to occur chiefly in carcinomata and the bodies described above were commonest in sarcomata.‡ They

* *Brit. Med. Jour.*, 1890, vol. ii, p. 1356.

† Touton, Virchow's *Archiv*, Bd. cxxxii, p. 427, 1893.

‡ Lubarsch states that the fuchsin bodies occur occasionally in sarcomata.

are not probably related to eleidin granules or to keratohyalin, as the epithelial cells of the skin treated by the methylene-blue method show none of them. Whether or not they correspond to the granule-like forms which have been described within certain cells of sarcomata after fixing in the fluids of Flemming, Zenker and Altmann I cannot be sure, as the descriptions given in the literature are very meagre. I have studied these sarcomatous tissues before the stain had taken effect, but could make out none of the granular bodies in the cells. It must remain doubtful then whether they represent pre-formed structures within the cell body or are to be looked upon as precipitates, possibly of nucleo-albumins, carried down by the methylene-blue. The same difficulty has been encountered by Held* in his studies of the so-called Nissl bodies within the protoplasm of nerve cells, but he concludes that the appearance of the blue masses in them is due to the precipitating effect of the dye.

FIBRES OTHER THAN NERVES WHICH TAKE THE BLUE STAIN IN TUMORS.

In the sections of sarcomata and carcinomata treated by this method a considerable number of fibrils which have a special affinity for the blue dye have been met with. In carcinomata they are seen here and there in the fibrous stroma, which otherwise, with the exception of its Mastzellen, ordinarily remains entirely unstained. They appear as fine wavy deep blue fibres with smooth contours, and are often of considerable length. In sarcomata similar blue stained fibres can be seen running in among the cells. Here the fibres are often of great length, in one case passing more than 100 sarcoma cells, and as a rule they are very straight, though in some shorter wavy fibres are to be seen. Some of them at least branch and anastomose with others; some are quite coarse, while others are very fine. No one with much experience with the method could confound them with nerve axones. Leredde* has described basophilic fibres occurring in mycosis fungoides; but in the case of this disease (specimen No. 9), which I have

* Held, Beiträge zur Structur der Nervenzellen und ihrer Fortsätze. *Arch. f. Anat. und Physiol.* (*Anat. Abth.*), 1895, p. 396.

* Leredde, *Journal d. maladies cutan. et syph.*, August, 1895, p. 502.

examined by the methylene-blue method, none of the blue fibres so common in sarcomata and carcinomata were met with. The relation in which the fibres which stain in these tumors stand—indeed if any such relation exists—to the ordinary connective tissue fibrils can be decided only by the making of control experiments on the same tissues, fixing and staining by the special methods for studying white and elastic fibres and reticulum.

In the myxomata (Nos. 6 and 11) the methylene-blue has stained some of the cells and their processes beautifully. The processes appear as long slender wavy fibres. Most of the cells have two processes; some only one; others three or more. In tumor No. 13 fibres of greater length, often running through several fields of the microscope, stained intensely in the blue, especially at the peripheral parts of the section where the piece of tissue was most exposed to the staining fluid. These fibres run out from the poles of the somewhat spindle-shaped myxoma cells, and as far as can be judged from this method alone are integral portions of them. It would be unsafe to deny, however, the possibility of the fibres being distinct from the cells, as the latter may be simply closely attached to the former.

DESCRIPTION OF PLATE I.

Fig. 1.—A section of a sarcoma of the tibia (tumor No. 4) in which four fine, wavy, slightly varicose axis cylinders are seen stained a blue color by Lavdowsky's modification of Ehrlich's method. Fixed by the method of Bethe; counterstained with alum cochineal. One fibre is seen dividing into two at this point. A small artery and vein are visible adjacent to the nerve fibres, all running in different directions and entirely surrounded by sarcoma cells. The nerve and blood vessels are evidently integral parts of the tumor. Three cells are to be seen which contain within their protoplasm the bodies or substances which stain blue, and show more highly magnified in Fig. 2.

Fig. 2.—Intracellular bodies from the mixed celled sarcoma of the tibia which stained by the blue of the same method. Various forms are shown: the small solid-looking spherical bodies and the larger vacuolated and crescent-like forms. The cells containing them are seen to be larger, swollen, somewhat irregular in shape, and contain feebly staining protoplasm.

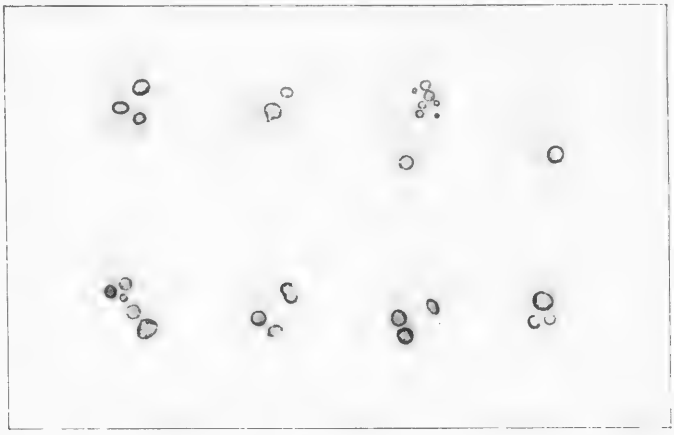

FIG. 2.

FIG. 1.

ON THE RELATION OF THE VOLUME OF THE CORONARY CIRCULATION TO THE FREQUENCY AND FORCE OF THE VENTRICULAR CONTRACTION IN THE ISOLATED HEART OF THE CAT.

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Plates II and III.

In the experiments recorded in this paper, the heart of the cat was kept beating by passing defibrinated cat's blood from the aorta through the coronary vessels into the right auricle and ventricle, whence it escaped through a cannula in the pulmonary artery. The blood thus transfused through the coronary vessels into the right heart flowed from the tube in the pulmonary artery in drops, as a rule. The volume of the transfused blood was measured by counting the drops. The volume could be increased or diminished at will by increasing or diminishing the pressure of the blood at the mouths of the coronary arteries. A Hürthle membrane manometer, connected with the left ventricle by a tube passed through the left auricular appendix and mitral valve, measured the force and frequency of ventricular contraction, the left ventricle and the connecting tubes being filled with normal saline solution. The experiments were performed in a warm chamber at a sufficiently constant temperature.

The results of the investigation show that the force of ventricular contraction is immediately affected by a change in the amount of blood supplied to the cardiac muscle, the ventricle beating with more force if the volume of the coronary circulation is increased, and with less force if it is diminished; and this holds true within wide, but, as yet, not accurately determined limits. The frequency of ventricular contraction, on the contrary, is largely independent of the blood supply to the cardiac muscle, and may remain almost unchanged

even while the force of contraction, in consequence of lessening the supply of blood, is much reduced. We noted also that the left ventricle of the isolated cat's heart, working with a constant load of a very few millimetres of mercury, will develop a high intracardiac pressure on a surprisingly small coronary circulation.

Certain other facts of interest were incidentally observed. The distension of the left auricle and ventricle lessened the coronary flow. The fibrillary contractions occasionally present sometimes gave place to wholly normal beats, one heart, we especially remember, showing strong fibrillary contractions during forty-five minutes and then falling into regular, normal contractions, which continued for more than an hour. The heart appears to add little, if any, fibrin to the blood.

Interesting also is the observation that a heart, from which the blood supply had been almost entirely cut off, began to group its contractions in a kind of "staircase" form. As the circulation through the cardiac muscle was restored, the individual groups in the intracardiac pressure curve grew longer and longer, until they gradually merged into an unbroken series of normal contractions.

It was remarked, further, that the amount of blood passing through the veins of Thebesius into the left heart was usually but a few drops per minute.

All these various observations will be discussed after the methods employed by us have been more fully described.

METHOD.

The method employed in our researches required a warm chamber; a blood-supply apparatus; a drop counter, for recording the number of drops flowing through the coronary vessels; and a membrane manometer, for recording the intraventricular pressure.

The warm chamber.—The warm chamber was made of "window sash," and was 200 cm. long, 80 cm. wide, and 150 cm. high, giving a contents of 2.4 cubic metres. Access to the chamber was afforded by two tightly closing doors, hinged above, which together formed the lower two-thirds of the front of the chamber. When the doors were raised, the entire length of the chamber was exposed, provid-

ing abundant room for arranging apparatus and for cleaning. The chamber stood upon a table, the top of which was made almost entirely of sheet copper. This sheet formed the roof of a copper tank, 150 cm. long, 60 cm. wide, and 15 cm. deep, partly filled with water. Thus the table was merely a support for the copper water-tank and the glass chamber over it. The water was put into the tank through an opening in the top, after which the opening was closed with a cork, perforated by a perpendicular glass tube, 15 mm. in diameter, which ran through the roof of the warm chamber and served to let out the steam when the water was heated. Thirty-two centimetres above the floor of the chamber this escape pipe was furnished with a short side branch, through which a part of the hot watery vapor passed into the warm chamber and kept the air moist.

The tank was heated by four Bunsen burners placed beneath it. It was our habit to light the Bunsen burners about an hour and a half before the animal was ready to be put in the chamber. At the end of that time, the temperature of the chamber having risen to about 37° C., the animal was placed as far as possible from the doors, one door was tightly closed, and the other was let down until it rested against the back of the observer in charge of the manometers, who stood with the upper half of his body inside the chamber. The temperature of the chamber remained sufficiently constant for our purposes, the large contents, namely, 2.4 cubic metres, and the large heating surface, 9000 square centimetres, keeping it so, in spite of the partial opening of one of the doors. The temperature was measured by a thermometer, the bulb of which hung in the middle of the chamber about 20 cm. above the animal. Many control readings were taken from a thermometer placed in the thorax. In the course of 27 experiments the thermometer was read about 500 times. The fluctuations during the period of observation were rarely over 0.5° C., an amount too small to constitute a serious source of error. In fact, our position in respect of temperature errors was not a difficult one, for our time of observation was made up of three brief, consecutive periods: the first, recording the force and frequency of ventricular contraction with a known volume of coronary circulation; the second,

with that volume altered in one direction or the other; and the third, with the coronary circulation restored, as near as might be, to the volume of the first period. All these periods together lasted but three or four minutes, sometimes less, and the temperature could vary but little during this short time.

Blood-supply apparatus.—The blood was contained in a glass tube, 14 cm. long, 3 cm. wide, of 50 cb. cm. contents, drawn out slightly at one end, and closed at the other with a rubber stopper. The drawn out or lower end connected, through a light brass stopcock, with the aortic cannula. The rubber stopper, closing the upper end, was perforated by two glass tubes. The first of these led through a stopcock to the filling reservoir, of the same dimensions as the blood reservoir but open at its upper end. The second of the tubes perforating the stopper of the blood reservoir was a short ⊢, the side branch of which opened, through a stopcock, to the atmosphere, while the direct tube led, through a stopcock, to the pressure bottle.

To fill the blood reservoir, all the stopcocks were closed and the defibrinated blood was poured into the filling reservoir. The stopcock leading to the atmosphere and then the stopcock leading to the filling reservoir were now opened and the blood filled the blood reservoir. The last-mentioned cocks were closed again, and those leading respectively to the aortic cannula and the pressure bottle were opened. The contents of the blood reservoir and its communicating tubes, namely, the aortic cannula, the aorta and the main coronary arteries, stood now under a constant pressure nearly that of the pressure bottle.

The pressure was made by joining the pressure bottle with a thick-walled rubber tube to a water bottle, suspended from the ceiling of the operating room. The water bottle could be raised or lowered and fixed at any height. It was so arranged that the level of the water within remained the same, whatever the height of the bottle. This constant level was maintained by an outflow tube in the shape of an ⊥. The branch of the outflow tube rose to a height of about 15 cm. above the level of the floor of the bottle, and then turned to be continued downward into a sink. At the turn it was provided with an upright tube, open to the atmosphere, to prevent siphonage. The

direct limb of the outflow tube of the water bottle was connected with the pressure bottle in the warm chamber. The water flowed continuously into the water bottle through the neck, and, passing through the tubulure, rose in the upright branch and turned down into the sink. A small part, however, found its way down the pressure tube to the pressure bottle. The pressure bottle being very large—its capacity was 5000 cb. cm.—and the volume of the coronary circulation very small, it could be used several hours without requiring to be emptied. With this apparatus the pressure at the mouth of the coronary arteries could be kept sufficiently constant at any desired level, or rapidly and easily changed simply by raising or lowering the water bottle. The flow through the coronary arteries could thus be varied within wide limits.

The pressure at the mouth of the coronary arteries was recorded by a mercury manometer connected with a cannula passed into the innominate artery at its origin from the aortic arch. In some experiments the manometer was furnished with a writing style. The curves thus secured (see Plate II, and Figs. 2 and 3, Plate III) bear witness to the uniformity of pressure at various levels and the ease with which the pressure is modified. The mercury manometer is also useful in determining whether a failure in the coronary circulation is due to lack of pressure or to an obstruction in the blood-vessels.

The drop counter.—The drop counter consisted of a glass tube, 165 mm. in length and of 4.5 mm. lumen, one end of which was tied in the pulmonary artery, just above the semilunar valves. The other end projected over the cat's left shoulder, and was usually somewhat lower than the heart, so that, when full, it acted as a weak siphon and favored the flow of blood. The same tube was used in every experiment. The blood dropped out of this tube on a triangular piece of thin aluminium about 17 mm. long and 14 mm. wide at the base, placed about 4 cm. below the outlet of the tube. The aluminium, which was so bent that the blood could not collect upon it, but ran off into a porcelain dish beneath, was fastened to the lever of an ordinary Marey tambour. From this ran a rubber tube to a small and very sensitive recording tambour, covered with very thin rubber, and

carrying a very light straw lever, which made a sufficiently large excursion for each drop that fell on the aluminium plate.

In a number of experiments the drop counter was not used, the drops being counted by an assistant, in periods of 15 or 30 seconds each. Another assistant marked the periods on the smoked paper by closing the circuit of a writing electro-magnet. An example of this marking is seen in Fig. 1, Plate III.

It will be remembered that the experiments we are discussing consist in comparing the frequency and the force of contraction in two or three consecutive short periods, while the conditions under which the heart is working remain the same, except that the volume of the coronary circulation is made to vary by varying the pressure at the mouths of the coronary arteries. The volume of the coronary circulation is estimated by counting the drops from the outflow tube. A difference in the size of the drops would, therefore, constitute a source of error. We must, then, consider the possible causes of such a difference. It might be thought that the size of the drops would vary with the quickness of flow, the more frequent drops being smaller than the less frequent ones. In this case we should expect that a given number of drops escaping from the outflow tube at a slow rate would differ in volume from an equal number escaping at a rapid rate. But the volume does not differ. On the contrary, the slow and the rapid drops are of approximately equal volume, as the following experiment shows.

Experiment, February 13, 1896.—Giving the volume of 100 drops of blood discharged at a slow and at a rapid rate, the variation in rate being produced by varying the pressure of the blood at the mouths of the coronary arteries, the temperature remaining unchanged.

Coronary Pressure.	Coronary Flow.	Volume of Coronary Flow.
18 mm. Hg.	100 drops.	8.25 cc.
18	100	9.00
18	100	8.75
60	100	8.25
62	100	8.00
62	100	8.25

It is seen at first glance that the volume of 100 drops is approximately the same at a coronary pressure of 18 and of 62 mm. Hg, in other words, with a slow and with a rapid flow. The difference in coronary pressure (44 mm. Hg) in this experiment is as great, lacking 1 mm., as in any experiment presented in this paper. It seems fair to conclude, then, that the difference in rate of flow, which in this experiment failed to influence the volume of an equal number of escaping drops, must, in the remaining experiments, likewise have failed to influence their volume.

This same experiment disposes of another speculative objection, namely, the possibility that strong contractions of the right ventricle dislodge larger drops from the outflow tube than weak contractions. Were this criticism sound, we should have a greater volume per 100 drops at a high coronary pressure than at a low pressure, for a rapid coronary circulation in consequence of a high aortic pressure causes the ventricles to beat more forcibly. But the measurements of February 13 demonstrate that the size of the drops is approximately the same, in spite of the inevitable difference in force of contraction entailed by a difference in coronary flow corresponding to a rise from 18 to 62 mm. Hg.

The criticism we have just answered has lurking within it the idea that the number of drops is, in some way, related to the number of heart beats. We have not found this to be the case. On the contrary, we shall show, when we come to discuss fully the outcome of our researches, that great changes may be made in the number of drops of outflow, with little and sometimes no change in the frequency of contraction.

Further information regarding the volume of the outflow drops is given by the record of the drop counter, which, as may be seen most clearly in Fig. 2, Plate III, records equal excursions for drops at slow and rapid rate of flow, and during strong and weak ventricular contractions.* Blood drops of unequal volume, being of unequal weight, would have caused unequal excursions of the drop-counting lever.

*An apparent exception to this statement is seen in Fig. 3, Plate III, in which the excursions are not everywhere equal, but the inequality is here due to unequal friction of the delicate lever of the recording tambour on the smoked paper. If the degree of friction is carefully regulated, the uniformity seen in Fig. 2 will be secured, *i. e.*, positive evidence of equality.

The membrane manometer.—The membrane manometer employed to record the pressure in the left ventricle was essentially that devised by Hürthle. The chamber was covered with thin rubber membrane. The writing lever rose 17 mm. above the atmospheric pressure line, when the pressure within the chamber was raised from zero to 100 mm. Hg. The manometer was usually placed on or near the level of the heart, the difference between the two levels probably never exceeding 4 cm. The tube connecting the manometer with the ventricle was about 15 cm. long and had 3 mm. lumen. It was passed through the left auricular appendix and mitral valve, the appendix being tied about it some distance from the circumflex branch of the left coronary artery.

The isolation of the heart.—The cats are put in a box, etherized until motionless, placed on a Czermak holder, tracheotomized, and, still under ether, bled from the left carotid artery until respiration ceases. The sternum, with the contiguous cartilages, are now rapidly removed, thus exposing the heart and the great vessels, and the left subclavian and innominate arteries, the superior vena cava, the right azygos vein, the inferior vena cava, and the aorta, beyond the origin of the left subclavian artery, ligated in the order named. Cannulas are now placed in the innominate artery and the aorta, and the outflow tube tied into the pulmonary artery just above the semilunar valves. The innominate cannula should be provided with a stopcock. These various operations occupy but a few moments, and are usually finished before the heart has entirely ceased to beat. The dead cat is now put in the warm chamber, and the aortic cannula coupled to the blood reservoir, already filled with the cat's blood, which, meantime, has been whipped, filtered through glass wool, and kept near its normal temperature in the warm chamber. The filling of the aortic cannula is best accomplished by allowing the blood to flow through it into the aorta and out again by the innominate cannula. This device expels the small clots that sometimes form above the semilunar valves and drives out the air bubbles as well, a matter of much importance, for embolism by blood clots or air bubbles is apparently the most frequent and dangerous of the causes which work against the survival of the

heart. As soon as the clots and air bubbles are out, the stopcock of the innominate cannula, and the stopcocks leading from the blood reservoir to the atmosphere and the filling reservoir, are closed, and the stopcock from the blood reservoir to the pressure bottle opened. The heart swells as its arteries are filled, and in a moment a dark venous stream is seen to flow out of the pulmonary artery, the heart, as a rule, beginning to beat in a vigorous and regular fashion. The innominate cannula may now be connected with the mercurial manometer. It is well to begin the experiment with a coronary pressure of not more than 50 mm. Hg, for at first the blood runs more freely through the coronary vessels, often coming out for a short time too fast to be counted. In a few minutes the flow diminishes. The pressure may then be increased.

The failure of the heart to beat, and even the presence of fibrillary contractions, should not discourage the operator, provided the circulation through the coronary vessels is fairly good. Strong fibrillary contractions may continue half an hour or more and still give place to good, coordinated beats. If the blood refuses to flow, even at a pressure of say 70 mm. Hg, a search should be made for blood clots at the mouths of the coronary arteries. Sometimes air emboli cause the failure. Usually in such a case the ramus descendens will be seen to be filled with air. Yet we have observed even such hearts begin to beat.

The left side of the heart is sometimes considerably distended. This distension has always, so far as we remember, lessened the coronary flow, a fact to which we shall presently return. The distension of which we speak may, of course, be the result of leakage through an incompetent semilunar valve. In that case the mercury manometer usually falls slowly. If the tube leading from the left ventricle is now opened, blood will escape from it, while the mercury manometer goes speedily down to atmospheric pressure. Almost always, however, the semilunar valves are intact. The mercury then remains stationary, when the tube from the left ventricle is opened, and the flow from the tube after the first moment is at the rate of a few drops per minute, which indicates that the distension of the left ventricle is the result of the gradual inflow from the veins of Thebesius. We

have invariably found that the quantity of blood thus escaping into the left side of the heart is too small to constitute a source of error in these experiments. We shall come back to this in the discussion of our observations.

Oxygenation of blood.—The oxygenation of the blood is best performed by shaking it in a beaker in the warm chamber. We made many trials to determine whether it was not better oxygenated by a stream of pure oxygen gas, but the decision was in favor of shaking. Perfect oxygenation grows apparently more and more difficult as the experiment continues.

The formation of *fibrin* in the blood gave very little trouble. After the contents of the cardiac vessels and the great arteries used in the method have been thoroughly washed out with defibrinated blood, and this is usually accomplished by the first 50 cc. transfused, filtration of the blood which has passed through the cardiac muscle is rarely necessary.

Finally, before passing to the discussion of results, we may add that the flow through the coronary arteries usually diminishes when the experiment is prolonged over an hour. Some temporary acceleration may then be gained by raising the coronary pressure, but often the highest pressure that the semilunar valves will safely bear fails to restore the rapid flow which marked the earlier stages of the experiment. It is obvious that many causes may contribute to bring about the increased resistance to the passage of the blood. Alterations in the physical properties of the blood, the increasing difficulty of its proper oxygenation, physical changes in the tunica intima of the arteries owing to disturbed nutrition, and constriction of the vessels are some of these. That narrowing of the vessels does actually take place in organs submitted to artificial circulation was long ago observed by Sadler (10), Genersich (3) and Heger (5), and referred to local asphyctic constriction. But we cannot discuss this question here.

Discussion of Results.

A number of the facts which it is the object of this paper to point out are illustrated by Plate II, the graphic record of the following experiment.

EXPERIMENT 9, MARCH 20, 1896.

The heart of a cat weighing 3012 grammes was kept alive by feeding it with defibrinated cat's blood, after the manner described under "Method," page 20. The curves reproduced in Plate II were taken shortly after the isolation of the heart. The temperature remained throughout the experiment at about 37° C., the variation being certainly not more than 0.5° C. The heart weighed 15.5 grammes. The upper curve (curve 1) in Plate II was drawn by a mercury manometer connected with the innominate artery at its origin; it gives the "coronary pressure," *i. e.*, the pressure in the aorta at the mouth of the coronary artery; curve 2 gives the pressure in the left ventricle; curve 3 the time in seconds; and curve 4 shows the volume of the coronary circulation, each elevation in the curve recording the outflow of one drop from the tube in the pulmonary artery (100 drops = 8.3 cc.); the horizontal line in this same curve 4 is the atmospheric pressure for the mercury manometer (curve 1). The numerical values of the curves are presented in Table I.

TABLE I.

Giving the Numerical Values of the Curves in Plate II.

Time in Periods of 15 secs.	Coronary Pressure at the close of each 15 secs.	Coronary Flow during each 15 secs.	Maximum Intraventricular Pressure at the close of each 15 secs.	Number of Contractions during each 15 secs.
1–15 secs.	90 mm. Hg.	14 drops.	53 mm. Hg.	28
15–30	90	12	53	28
30–45	64	10	47	28
45–60	54	6	41	28
60–75	52	5	36	28
75–90	52	5	35	28
90–105	52	4	32	28
105–120	52	4	30	27
120–135	52	5	28	28
135–150	52	4	24	27
150–165	60	5	21	27
165–180	80	10	18	27
180–195	88	14	22	27
195–210	88	16	25	27
210–225	88	16	32	27
225–240	88	16	38	28
240–255	88	16	52.5	28

Force and frequency of ventricular contraction.—Examining Plate II the reader will perhaps notice first the regularity of the curves; the steadiness with which the pressure in the aorta is maintained at the desired level; the ease with which it is lowered and then restored to its former plane; the absolute command of the volume of the coronary circulation; the way in which the number of drops flowing out of the pulmonary artery follows the rise and fall in coronary pressure; and the smooth, uniform action of the isolated, surviving heart—facts which create confidence in the method. Soon, however, the beautiful dip and rise of the intraventricular pressure curve will claim attention. The intraventricular pressure is seen to sink as the outflow from the pulmonary artery (curve 4) diminishes, and to rise again as the outflow recovers its former volume. The force of the ventricular contraction wanes as the supply of blood to the ventricular muscle lessens, and increases again with the increase of the food supply. Nor does the force of contraction follow tardily the changes in the food supply. The two go hand-in-hand. They are almost perfectly synchronous. A glance at the curves of Plates II and III shows that the rise and fall in the volume of the coronary circulation immediately alter the force of contraction.

The importance of this observation is, in our opinion, very considerable. It would seem *a priori* probable that an organ, charged with a task on the proper performance of which life itself depends, would have within itself a reserve of instantly available power that should make it independent of very temporary alterations in its food supply. This, however, is not the case. There is no such immediately available reserve. The force of cardiac contraction is most closely dependent on the quantity of blood supplied to the cardiac muscle.

This result is in entire accord with Porter's (9) observation of the lowering in force of contraction that follows the closure of a very large branch of the left coronary artery in the dog.

The frequency of contraction is little impaired by even great reductions in the food supply. In the example before us (Plate II) the volume of the coronary circulation was reduced 71 per cent; the frequency of contraction fell but 4 per cent, while the force fell 67

per cent. On restoring the coronary pressure to about its former level, the volume of the coronary circulation increased 75 per cent, causing a rise of only 4 per cent in frequency, but of 67 per cent in force of contraction.

An examination of the remaining experiments in which the intraventricular pressure was measured gives further weight to the view already presented. Table II (p. 26) contains the percentage variations in coronary pressure, coronary flow, intraventricular pressure, and frequency of contraction for twelve experiments, conducted at a constant temperature of about 37° C., according to the method already described. On the left-hand side the consequences of diminishing the volume of the coronary circulation are shown; on the right side, the consequences of restoring the volume to about its former level. The percentages are secured by dividing the fall or rise in coronary pressure, the number of drops per 30 secs., the intraventricular pressure, and the number of contractions per 30 secs. by respectively the coronary pressure, the number of drops, the intraventricular pressure, and the number of contractions observed before the coronary pressure was first changed. Thus, taking Experiment 9, page 23, as an example, the number of drops flowing from the coronary artery fell, when the coronary pressure was reduced, from 14 to 4 per 15 secs., a difference of 10, which is 71 per cent of 14, the rate of flow before lowering the pressure. The result of lowering the pressure was, therefore, a fall of 71 per cent in the volume of the coronary circulation, and this figure is accordingly set down in its proper place in Table II.

On glancing at the averages at the bottom of each column, it appears that the average fall and rise in coronary pressure, or driving pressure of the coronary blood, is practically equal, and that the average changes produced in the coronary circulation by lowering and raising the coronary pressure 41 or 42 per cent are also equal.

The average rise in intraventricular pressure and frequency of contraction is a little less than the average fall. This discrepancy does not mean that the recovery of force and frequency after the restoration of the coronary circulation to its former value is imperfect. Recovery within the wide limits used by us has always been complete,

even in the occasional instances in which the heart-beat became irregular, as illustrated in Fig. 3, Plate III. The lower averages for the rise of intraventricular pressure and frequency of contraction are due simply to the fact that a curve long enough to show full recovery could not always be written within the length of a Baltzer drum, and the use of a larger kymograph in the warm chamber would have been very inconvenient. Thus it happened more than once that the clock-work had to be stopped a half-minute or so before the curves had attained their former level. We would not give the impression that the recovery is slower than the fall. Careful examination of all the curves show that the heart responds quite as rapidly to a restoration as to a diminution of its blood supply.

TABLE II.

Comparison of Percentage Variation of Coronary Pressure, Coronary Flow, Intraventricular Pressure, and Frequency of Contraction.

Number of Experiment.	FALL.				RISE.			
	Coronary Pressure.	Coronary Flow.	Intra-vent. Pressure.	Frequency of Con-traction.	Coronary Pressure.	Coronary Flow.	Intra-vent. Pressure.	Frequency of Con-traction.
1	36 %	69 %	50 %	9 %	36 %	63 %	40 %	6 %
2	42	75	58	3	*	..	..	..
3	45	73	53	17	47	80	50	7
4	45	85	57	10	44	81	46	9
5	45	86	67	13	45	88	62	10
6	44	75	59	7	45	74	42	5
8	30	70	11	..	30	73	38	..
9	42	71	67	4	41	75	67	4
10	44	77	58	8	43	77	52	8
11	42	63	45	14	40	69	54	14
12	44	68	21	6	43	50	21	4
13	45	64	38	12	*	..	..	..
Average	42	73	49	9	41	73	47	7

*The rise of Exp. 2 and Exp. 13 has been omitted because the smoked surface employed for the curves of these experiments was used up before the heart had time to recover from the lessening in the volume of the coronary circulation.

The average reduction in intraventricular pressure is almost 50 per cent, but in frequency of contraction only 9 per cent, certainly a striking difference. In one experiment, indeed, the force of contraction fell 11 per cent and then rose 38 per cent, while the frequency

remained unchanged. This, however, must be regarded as exceptional, for the record in this case was made toward the end of a long series of experiments, after the heart had lost much of its sensitiveness to changes in its blood supply.

Special attention should, we think, be drawn to the experiment just mentioned, in which, in a heart which had long been under the experimenter's hands, a reduction of 70 per cent in the volume of the coronary circulation caused a fall of but 11 per cent in the force of contraction, and no fall whatever in the frequency of contraction. Those familiar with the literature concerning the closure of the coronary arteries in the dog will be interested in the bearing of this fact on Cohnheim and von Schulthess-Rechberg's observation, that ligation of the coronary arteries in the dog stops the heart more speedily and with greater certainty, when the operation is quickly done, than when a longer time elapses between the exposure of the heart and the interference with its blood supply.

Refilling the blood reservoir with blood has a favorable influence on the force of contraction, probably not to be explained as a reaction from an hypothetical depression caused by the very brief and inconsiderable fall in coronary pressure which refilling the reservoir entails. Cyon (2) long ago pointed out a similar phenomenon in the surviving heart of the frog. In a few moments after refilling, the heart reaches what may be termed an equilibrium, beating then with great uniformity. Fig. 2, Plate III, shows at the extreme left the rising intraventricular pressure immediately following a fresh charge of blood. Fig. 1 of the same plate illustrates the uniform action soon attained.

Distension of the heart.—Turning from the main objects of the investigation, namely, the relation between the volume of the coronary circulation and the force and frequency of ventricular contraction, to the phenomena incidentally observed, we shall deal first with the effect of distension of the cavities of the heart on the flow of blood through its walls. It not infrequently happens in the first five minutes after the feeding of the isolated heart begins that the left auricle and ventricle become distended with blood. The first thought would naturally be that the aortic valves were not perfectly tight, thus allow-

ing some of the blood kept at a high pressure in the aorta to enter and distend the ventricle. But insufficiency of the cat's aortic valves to a pressure of not more than 100 mm. Hg is rarely observed, and can easily be excluded by the device already mentioned on page 21.

A part, if not the whole, of the distending blood comes from the veins of Thebesius (13), as will be shown in the next paragraph. We wish first to point out that the distension of the cavities of the heart with blood always, so far as we remember, diminished the flow through the coronary vessels. The consequences of such distension are now making the subject of a special study, so that we shall no more than point out the probable importance of this observation to the pathology of the heart. It cannot be doubted that the pressure of the blood in the cardiac capillaries and veins is very low. As the distension of the heart witnessed in our experiments was sufficient to diminish the coronary flow, it is probable that the distension observed during excessive muscular effort, and in many pathological conditions likewise, diminishes the supply of blood to the heart muscle. The speedy consequence of such a falling off in the coronary circulation is a lowering of the force of contraction, and this lowering must still further reduce the amount of blood flowing through the stretched heart walls. It is not unlikely that many of the heart "failures" ascribed to "paralysis of the heart by over-distension" are in reality failures due to ischæmia of the heart. But it would be unwise to speak too decidedly on this question pending the results of the experiments now making.

Veins of Thebesius.—It has already been said that a part of the blood sometimes found in the left heart after the beginning of the artificial coronary circulation comes out of the veins of Thebesius. The proof of this is that blood continues to flow into the left heart after every source except these veins has been shut off. The quantity thus entering the left auricle and ventricle is, however, very small; far too small to affect the method employed in this paper. The amount can be readily observed by draining the left heart by a tube passed through the left auricular appendix. Such a tube was in the ventricle throughout the experiment of February 26, in which the isolated heart beat 4 hours and 12 minutes. The flow was scarcely measureable. During

the experiment of February 28 not more than three drops per minute flowed from the cannula in the left ventricle, while the flow from the right side of the heart was from 40 to 145 drops per minute (3.3 cc. to 12 cc.), the pressure at the semilunar valves being made to vary from 36 to 68 mm. Hg. Similar observations were often made. It was noticed also, during the recording of intraventricular pressure, that when the left heart filled, the base line of the membrane manometer curve gradually diverged from the atmospheric pressure line. A base line that remains parallel to the atmospheric pressure line excludes any significant addition to the contents of the left ventricle. It will be seen that the curves of Plates II and III show this parallelism. It is hardly to be doubted that the flow into the left heart from the veins of Thebesius is somewhat greater during life than in the surviving heart, inasmuch as there is little doubt that the coronary circulation as a whole is larger during life.

The smallness of the blood supply on which a good intraventricular pressure and a regular beat can be kept up is surprising, even when the small load of the left ventricle (a few mm. Hg) is taken into account. Reference to Fig. 1, Plate III, will show that admirable contractions were secured with 40 drops per minute (about 3.3 cc.). In the experiment of March 13 fair contractions were seen with a coronary circulation of only 22 drops per minute (less than 2 cc.). This heart had been isolated a long time when the observation was made. The ordinary volume during our experiments ranged, for vigorous contractions, from 3.5 cc. to 13 cc. per minute.

Fibrin in the blood.—A noteworthy incident in the beautiful method of isolating the lungs and heart, devised by Ludwig and Stolnikow (11), and afterward employed by Pawlow (8) and others, is the freedom of the circulating blood from fibrin. One would suppose that blood driven through the rubber and glass tubing of the new Ludwig Stromuhr used by these observers would speedily clot, but it is found that the circulation through lungs, heart and Stromuhr goes on for several hours. Either the heart makes no fibrin factors, or, if made by the heart, they are destroyed, probably in the lungs. Such at least are the ideas at present entertained regarding the failure of the blood

to clot. With the wholly isolated heart shut off, as it is, from even the lungs, valuable information can be added to the observation of Ludwig and Stolnikow. We have repeatedly noticed that after 50 to 100 cc. of defibrinated blood had passed through the coronary vessels of the cat's heart, it was no longer necessary to filter the blood. The early filtrations were required because of the fibrin derived from the unwhipped contents of the cardiac vessels at the beginning of the experiments. Whether no fibrin factors are formed in the metabolism of the heart, or whether the amount so formed is too small to affect the circulation, cannot be stated without quantitative experiments, which, we are informed, will soon be published.

The "staircase" beats sometimes observed when the coronary circulation is very much reduced are illustrated by Fig. 3, Plate III. It is noteworthy that when the blood supply is restored, the entire "staircase" is, so to speak, lifted, each of the contractions being more forcible than before, but the group as a whole preserving the staircase character. Interesting, too, is the strong compensatory contraction following each dropped beat.

Fibrillary contractions.—The recovery of the cat's heart from fibrillary contractions has already been mentioned on another page. We return to the subject here in order to point out how irreconcilable our observations are with the view that fibrillary contractions are occasioned by mechanical injury of the heart. We have seen normal contractions give place to fibrillary contractions in consequence of disturbances in the coronary circulation, although the heart had meanwhile not been touched.

Our observations, finally, are also opposed to the idea that fibrillary contractions are necessarily fatal in the dog. We have more than once witnessed marked and long-continued fibrillations in the cat's heart yield to normal contractions, prolonged for many minutes, and we cannot admit that the heart of the cat is so fundamentally different from the heart of the dog that a disturbance frequently recovered from in the former should be always irrecoverably fatal in the latter. None of the investigators who assert that fibrillary contractions are necessarily fatal in the dog has published experiments in which the

resuscitation of the dog's heart after fibrillary contractions has been attempted by the only rational plan, namely, the re-establishment of the coronary circulation (12).

In conclusion, we desire to express our thanks for the advice and unstinted aid of Dr. W. T. Porter, at whose suggestion this work was done.

HISTORICAL NOTE.

The maintenance of the contractions of the heart by means of the artificial circulation of defibrinated blood through the coronary arteries was first accomplished by Martin and Applegarth (7) in their experiments on "The temperature limits of the vitality of the mammalian heart." The essential principle of their method has been adopted by all subsequent investigators, namely, Arnaud (1), Hédon and Gilis (4), Langendorff (6), Porter and ourselves. It is described by Martin and Applegarth as follows: All the branches of the aorta, except the coronary arteries, are ligated. The venæ cavæ are also ligated. In the aorta itself is placed a cannula, which is connected with a Mariotte's flask, raised a sufficient height above the organ. The defibrinated blood from the flask fills the connecting tubes, the aorta and the coronary arteries at a constant pressure, which, of course, is quite independent of the force and frequency of the heart beat. The blood taking the coronary circuit, on reaching the right auricle, proceeds to the corresponding ventricle, and from it through the lungs to the left auricle. This blood is, therefore, the only blood entering the cavities of the heart or passing through the lungs, unless there be some inefficiency of the aortic semilunar valves. That the cavities of the heart are not distended with more blood is found not to influence the normal character of its beat, which continues rhythmically and forcibly for three or four hours.

Arnaud, in 1891, apparently unaware that his observation had been anticipated by Martin and Applegarth, also revived the rabbit's heart by injecting defibrinated blood into the aorta.

Hédon and Gilis, the following year, performed the same experiment on a dog and on the heart of an executed man. They make no mention of previous experiments.

In a recent work on the isolated mammalian heart, Langendorff, after citing the experiments of Arnaud and of Hédon and Gilis, remarks in an appendix that he has purposely omitted any reference to Martin's work, because the method of Martin and his pupils has very little in common with his own. It is plain, however, that the distinguished physiologist of Rostock has seen only the earlier works of the Baltimore school. He nowhere mentions the investigations of Martin and Applegarth, and refers only to the previous researches of Martin and his associates, in which blood was supplied to the heart through the right auricle.*

The methods of Martin and Applegarth and of Langendorff are alike in the following fundamental points: (1) The heart muscle is fed by maintaining a constant pressure in the root of the aorta, and hence in the coronary arteries, the nutrition of the heart muscle being not directly dependent on the contractions of the heart. (2) The right ventricle receives no blood except the small quantity coming from the coronary veins. (3) The left ventricle works against a constant resistance. (4) The right ventricle in Langendorff's hearts works against practically no resistance. In the hearts of Martin and Applegarth the resistance can be little more, for the pulmonary circulation is reduced to the blood passing through the coronary vessels, and our own observations show that this quantity is but a few cubic centimetres per minute. (5) In both procedures, probably, the influence of the central nervous system is excluded, for we can hardly suppose that the brain and spinal cord remain functional after the total arrest of the circulation.

The method employed in the present investigation is also a modification of that used by Martin and Applegarth.

The first account of the intracardiac pressure in the isolated heart and the effect of changes in the volume of the coronary circulation on the force and frequency of the ventricular contraction was published by Porter in The American Text-book of Physiology, Philadelphia, 1896, p. 476, Fig. 125.

* Martin and Applegarth's work is indexed by the *Index Medicus*, but is omitted in the *Centralblatt für Physiologie*, the *Jahresbericht über die Fortschritte der Physiologie*, and Tigerstedt's Lehrbuch der Physiologie des Kreislaufes.

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12. For the literature of this subject see Porter, *The Journal of Experimental Medicine,* i, 1896, p. 46 *et seq.*

13. Compare Gad's note on the *foramina Thebesii, Arch. für Physiologie,* 1886, p. 382.

DESCRIPTION OF PLATES II AND III.

Like curves have been given the same number in both plates and all figures.

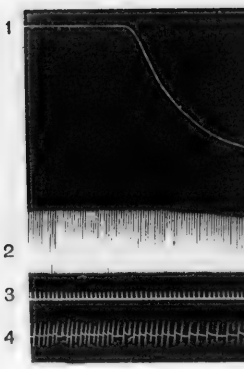
Curve 1 is everywhere the line drawn during the experiment by a mercury manometer connected with the root of the aorta. It indicates the blood pressure at the mouths of the coronary arteries.

Curve 2 is everywhere the intraventricular pressure curve, drawn by a Hürthle membrane manometer, connected with the left ventricle through the auricular appendix and mitral valve.

Curve 3 is time in seconds.

Curve 4 is the quantity of blood passing through the coronary vessels into the right side of the heart and thence out through a tube placed in the pulmonary artery. In Fig. 1, Plate III, the number of drops is given per 30 seconds, the number being respectively 20, 14, 5, 5 and 19 drops, and the periods of 30 seconds are registered by an electro-magnetic signal, while the drops are counted by an assistant. In the remaining figures of both plates the number of drops is registered automatically by the drop counter described on page 17. Each upward stroke in the curve records one drop. One hundred drops equal 8.3 cc. cat's blood. In Fig. 3, Plate III, and to a much less degree in Plate II, the friction of the writing point of the very sensitive recording tambour of the drop counter was too great, leading to irregularities in the height of the individual strokes. This difficulty is overcome in Fig. 2, Plate III.

Curve 5 is the atmospheric pressure for the mercury manometer (curve 1), drawn immediately after the experiment.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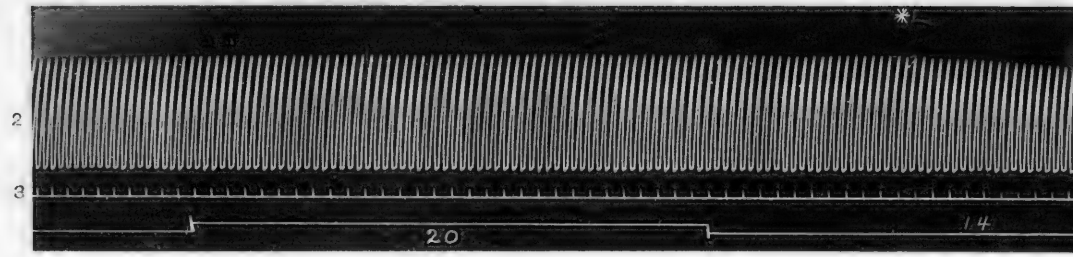
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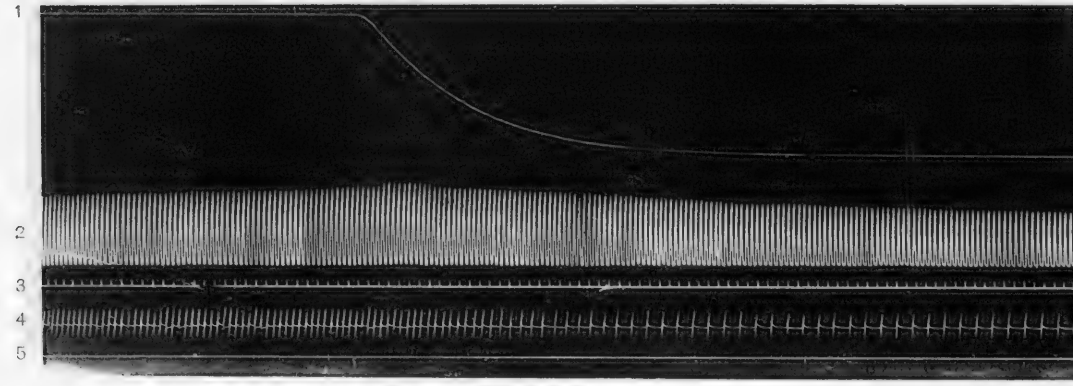
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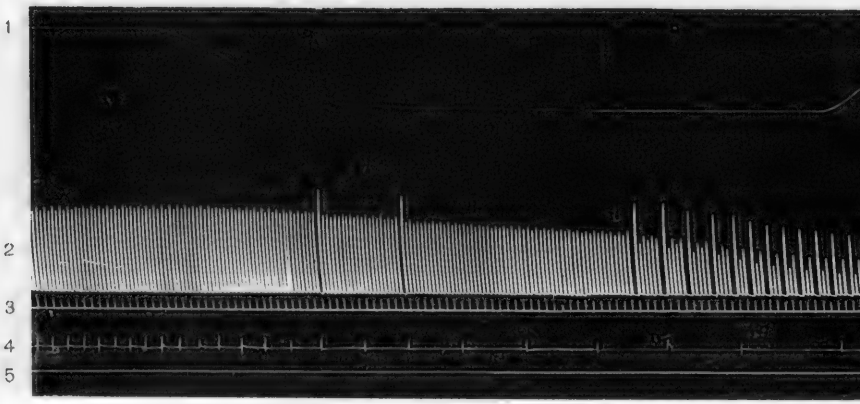
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FIG. 1.

FIG. 2.

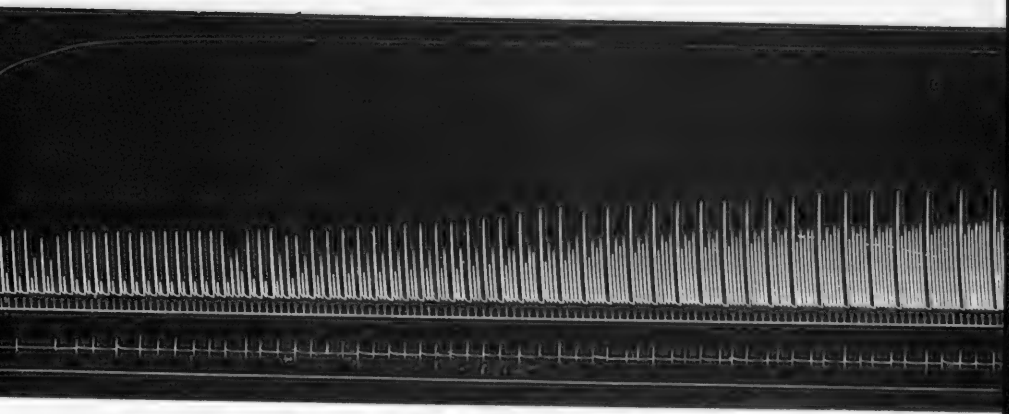
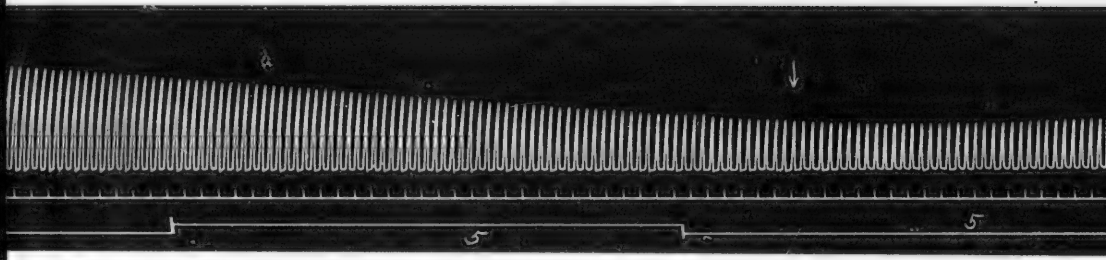


FIG. 3.



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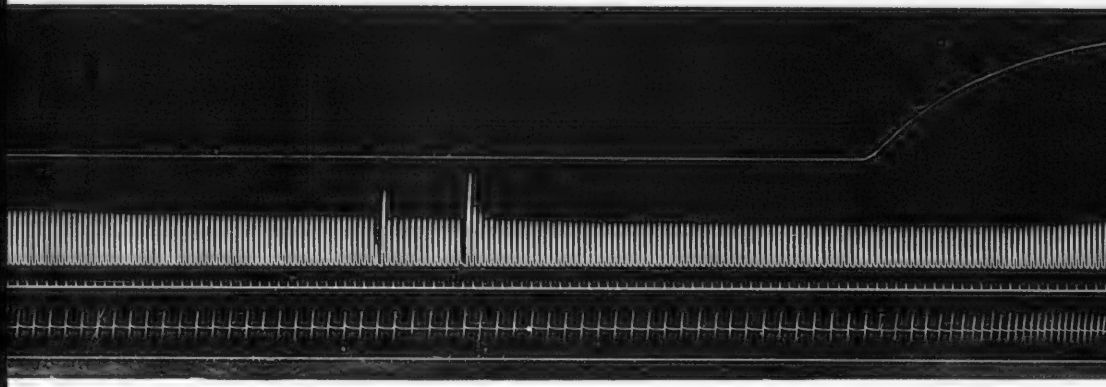

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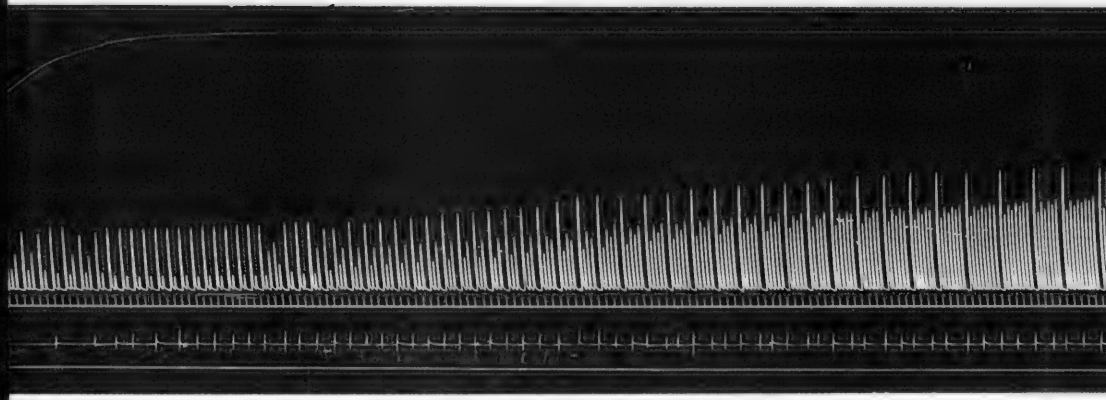

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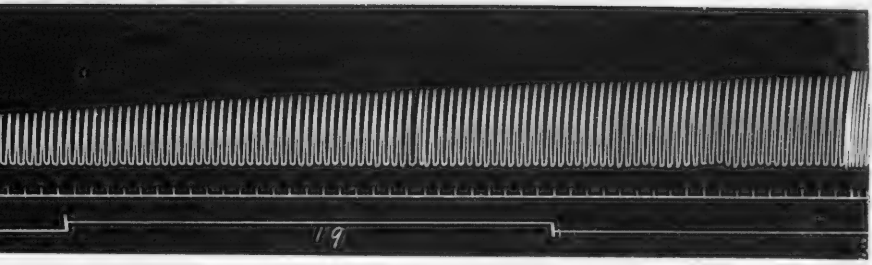
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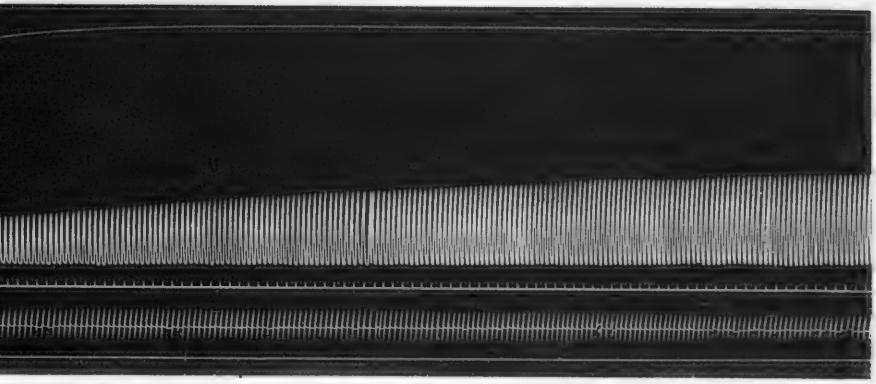
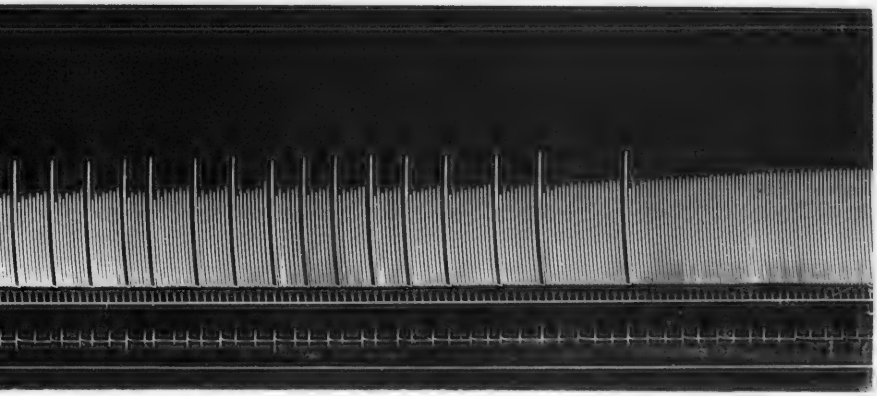




ON THE TIME OF REFLEX WINKING.

By DAVID P. MAYHEW, Ph. B.
(From the Physiological Laboratory of the University of Michigan.)

Plates IV-VIII.

The question of the time required by the central nervous system for the performance of simple reflex acts was brought up by a discussion as to the character of the knee jerk. The argument that the time required for that movement is less than it should be, were the cells of the central nervous system involved, did not seem satisfactory. The calculations leading to this conclusion were based on the well known experiments of Exner on the time of Reflex Winking,* the only undoubted reflex time yet ascertained for man, in which, by a very simple mechanical device, he measured the time occupied by a wink occasioned by the stimulus of an induction shock.

For the benefit of those to whom Exner's paper is not wholly familiar, it may not be amiss to give a brief résumé. In his apparatus a long, light lever was fastened to a frame so made as to be firmly held between the teeth of the subject. A thread fastened close to the pivoted end of the lever, after passing over a couple of pulleys, was glued to the eyelid by a bit of plaster. This of course transmitted the movements of the eyelid to the lever, which was made to write on a revolving drum. The stimulus employed was an electrical shock applied to the other eyelid. He varied the intensity of the stimulus by increasing or decreasing the distance of the secondary coil of his induction apparatus from the primary.

His results showed that the time occupied was less for a stronger than for a weaker stimulus. The following are his results as published:

* Pflüger's *Archiv*, viii, p. 526, 1874.

Induction coil. 9 cm. distant.	Induction coil. 5 cm. distant.
0.0600	0.0561
(0.0310)	0.0542
0.0716	0.0444
0.0678	0.0581
0.0678	0.0600
0.0658	0.0581
0.0774	0.0581
0.0600	0.0600
0.0929	0.0600
	0.0600
	0.0619
	0.0523
	0.0693
0.0662—mean.	0.0578—mean.

These figures represent, in fractions of a second, the total time occupied by the reflex movement, including, therefore, the time of transmission of the nerve impulse and the latent period of the muscle, as well as the true reflex time, or that taken up by the cells of the central nervous system. To obtain the latter it is necessary to subtract from the total times as given, the time taken by the first two mentioned functions. The entire nerve distance from the eyelid to the centre controlling its movements, which is in the calamus scriptorius, and return is 35 cm., and the time required for the nerve impulse to traverse that distance (taking the rate of transmission to be 62 m. per second) he calculated to be 0.0057 second. The latent period of man's muscle he estimated to be 0.005 second, so the sum to be subtracted from the total times is $0.0057 + 0.005 = 0.0107$ second.

In an article in the *Journal of Physiology** Waller makes the statement that he has verified these results. Aside from this statement, Exner's paper contains all that is known to the author upon the eyelid reflex.

APPARATUS AND METHOD.

As Tigerstedt by the introduction of electrical devices had lowered the latent period of frog's muscle from about one-hundredth to four one-thousandths of a second, it did not seem at all unlikely that similar

* Vol. xi, p. 387.

means would produce a like result in the time of reflex winking. An apparatus was therefore devised in which electric signals should take the place of the long lever of Exner. As the requisites for such an apparatus were of course lightness and convenience combined with great accuracy, the following method was adopted.

The eyelid as it closed in the act of winking was made to break an electrical circuit, and through a Desprez signal write on a revolving drum. To that end a small piece of platinum foil, bent at right angles in the form of the letter L and connected with a very fine copper wire, was fastened to the eyelid by a small strip of sticking plaster. It was insulated from the eyelid by a piece of rubber membrane fastened to its under surface. This bit of foil and its attachments were so light as to be scarcely felt by the subject. Indeed it was not felt at all after the first moment of application, and produced no inconvenience whatsoever.

Upon a very light aluminum frame (see Plate IV, Fig. 1) fitted to the head was mounted an adjustable bit of platinum wire (b) in such a manner as to touch the foil on the eye when the latter was opened. The wire being fastened to the head, followed its movements and maintained constantly its position relative to the eye. The wire on coming in contact with the foil completed an electrical circuit, which was broken by the slightest movement of the eyelid.

As contact or touch stimuli are the ones which normally excite this reflex, it was decided that a slight blow should be used as a means of stimulation. The following instrument was therefore devised: A small spring hammer (Plate IV, Fig. 2, a) was held back in position ready for striking by an electro-magnet. When it fell it struck a hard rubber plunger (c) which passed through a brass breech-block (d) soldered to a sliding brass strip attached to the frame of the device. The plunger was made flush with the outer surface of the block, and was intended to transmit the blow to the face. The end of the plunger was flat, so that the light blow produced no pain. The stimulus therefore was tactile. The tension of the spring of the hammer was regulated by a screw (b) at its fixed end, while the length of its blow was changed by sliding the brass strip, on which the breech-block was

fastened, along on the frame. The plunger was kept in its normal position by a spiral spring, within the hollow of the breech-block, which pressed a brass pin (e) running through the plunger against the breech-block.

The instant of the blow of the hammer was recorded as follows: An electrical current was sent through the breech-block (d) and thence into the pin, which limited the backward movement of the plunger, and out through a fine wire to binding post (g). When the hammer fell it forced down the plunger and thereby separated the brass pin (e) from the breech-block, thus breaking the electrical circuit for a brief moment at the instant the blow was delivered. Within one one-hundredth of a second the circuit would be restored by the pin being pushed back to place by the spiral spring. The stimulator was placed in the same circuit as the platinum foil on the eye, in which circuit was also a Desprez signal. The way in which the stimulator was applied to the face is shown in Plate IV, Fig. 1.

The operation of the apparatus in detail was as follows: When the hammer fell in giving the stimulus it struck the plunger, forcing it down, thereby delivering the blow and breaking the circuit at the same instant. The pointer of the signal then rose, but fell again at once, as the circuit was completed by the plunger returning to its normal position. Next the eyelid would fall reflexly, on account of the blow, and the circuit being again broken, the pointer of the signal would rise once more. If the pointer were writing on a revolving drum the tracing would be as follows: *a* , ~ *b*

a = rise due to blow of hammer, *b* = rise due to movement of lid. A tuning fork left its record through another signal just below this curve.

The recording drum (see Plate V) was placed with its axis horizontal. The Desprez signals (A) were mounted on a horizontal shaft, on which was also fixed an arm bearing a key (e) through which flowed the current to the electro-magnet of the stimulator. A band of rubber raised the signals from the drum, against which a slight pressure of the hand brought them. Their movement was limited by a rod on the shaft which, when the signals were sufficiently de-

pressed, struck against the frame on which the shaft was mounted. An arm on the shaft of the drum struck the key (e) of the electro-magnet circuit as the drum revolved, and by breaking the circuit allowed the hammer of the stimulator to fall.

To get the record the operator pressed down on the arm bearing the stimulator key, thus bringing the signals against the surface of the drum and the key within reach of the arm on the drum shaft. Waiting until the proper moment, he revolved the drum by hand and held the signals down just long enough for the key to be struck by the arm, then the pressure was released and the signals were raised by the rubber band. In this way the whole operation was practically noiseless, and was accompanied by no jarring whatsoever. It made no difference whether the speed of the drum were the same for each experiment or not, as each record had its own tuning-fork curve.

In practice the subject was seated on a stool with his head leaning against a solid head-rest. The fine adjustment at the eyelid was made by having the subject look at a block which might be raised or lowered. As the eyelid was found to follow, to a considerable extent, the vertical movements of the eye, this method was very delicate. If the subject looked at the same object in the same position, during each experiment, his eyelid would come back to the same position each time, and hence, unless the head-piece had been disarranged, no further adjustment was necessary.

In most of these experiments the stimulator was applied to the face about an inch below and to the outer side of the outer canthus of the eye, on the side from which the record was taken.

The recording apparatus at which the operator sat was placed in another room with the door closed. By observing the signals it could be learned whether the connections were right or not, and also whether the subject was winking or not, as each time he did so the pointer rose. Waiting until the subject completed one of his natural winks, the operator revolved the drum and got the record. The limit of error of the apparatus was very small, as the latent period of the signal affected the curve both when the blow was struck and when the subject winked, and hence served to correct itself. The

signal used was a very fine one of Desprez pattern. Its latent period, as was shown by numerous tests, varied only by one or two one-hundred-thousandths of a second, which may be disregarded.

It was very soon seen as the experiments proceeded that the results were not uniform. Thinking that this might be due partly to errors of the apparatus, the latter was tested from time to time as thoroughly as was possible, and always with the same result; the variations due to this source were never greater than might be attributed to variations in the latent period of the magnet, which, as above stated, were so small as to be disregarded.

The reading of the curves was done in the following manner: In the first place, the distance between the record of the blow and that of the movement of the eyelid was measured as accurately as possible with a rule graduated in one-hundredths of an inch and with the assistance of a magnifying glass. Then the tuning-fork curve was measured. The most convenient and accurate method of doing this was found to be as follows: The zero mark on the scale was placed opposite to one of the points on the tuning-fork curve, and another point which exactly coincided with some tenth-inch mark on the latter was sought. The distance from the zero point to this mark was noted, as well as the number of curves between. An example as recorded in the note-book is given: R. T. = 21.4 17 T. F. = 48. In this R. T. is the length of the curve made by the signal in the eye circuit, which shows the reflex time in tenths of an inch. T. F. is one tuning-fork curve, equal in value to $\frac{1}{200}$ of a second. A simple proportion gives the value of R. T. in fractions of a second, thus:

$$\text{R. T.} : 17 \text{ T. F.} :: 21.4 : 48,$$

or substituting value of T. F.,

$$\text{R. T.} : \tfrac{17}{200} :: 21.4 : 48,$$

which being solved gives R. T. = 0.0378 second. From this was deduced a rule which saved much figuring, viz.: Multiply value of R. T. in tenths of an inch by $\frac{1}{2}$ the coefficient of T. F., and divide by the value of T. F. in tenths of an inch. The quotient equals value of R. T. in one-hundredths of a second. If, on examining the tuning-fork curve, it was found when zero point of rule was adjusted to a

point on the former that no other point would coincide with a tenth-inch mark, ten of the tuning-fork vibrations were accurately measured with the aid of a magnifying glass and the result treated as before, thus R. T. = 22. 10 T. F. = 29.95. Solved according to rule given, $22 \times 5 \div 29.95 = 0.0367$.

The limit of error in the readings by this method was demonstrated to be within 0.0003 of a second. In other words, the first two figures of the result are absolutely accurate; the last may vary a little.

In a few cases, owing to oxidation of the mercury in the interrupter of the tuning fork, the curves made by the signal in that circuit were irregular. Instead of coming to a point at the top of the curve, some of them would round over and the curve would look like this.

In all such cases the curves were carefully stepped with a pair of delicate dividers, beginning at one pointed curve (as a) and carrying the stepping until another, or better, several other points at some distance from the first were exactly met by the dividers. The curve thus marked was easier to read and more accurately measured than ordinary curves not thus marked, so all of the curves of the last six hundred experiments were stepped, whether regular or irregular.

RESULTS.

The average duration of a reflex wink being desired, it was obviously necessary to take records from a number of men. The following table of 450 experiments was compiled from the records of sixteen subjects, male and female, all of which were taken under as nearly the same conditions as possible, the stimulus being the same for all.

TABLE OF 450 REFLEX WINKS.

.028	.029	.030	.031	.032	.033	.034	.035	.036	.037	.038	.039	.040	.041	.042	.043
.029	.030	.031	.032	.033	.034	.035	.036	.037	.038	.039	.040	.041	.042	.043	.044
2	2	2	6	5	9	18	15	25	18	29	29	35	30	28	36

.044	.045	.046	.047	.048	.049	.050	.051	.052	.053	.054	.055	.056	.057	.058	.059
.045	.046	.047	.048	.049	.050	.051	.052	.053	.054	.055	.056	.057	.058	.059	.060
32	21	19	17	22	15	13	5	5	4	2	2	2	1	0	1

The figures in the second horizontal column show the number of records which fell between the limits of time given directly above in the first horizontal column. Thus between .028 and .029 second there were two records. between .043 and .044 second there were thirty-six.

The average of these four hundred and fifty experiments is 0.042 second. Such an average is of course misleading, as most of the records fall either above or below this time. It is, however, worthy of note that, with but two exceptions, all of the results fall below the shortest average time given by Exner.

This is seen still more strikingly in the following table, which embraces the preceding, and which was compiled from all the results taken with the same stimulus under *all* conditions.

TABLE OF 1056 REFLEX WINKS.

.028	.029	.030	.031	.032	.033	.034	.035	.036	.037	.038	.039	.040	.041	.042	.043
.029	.030	.031	.032	.033	.034	.035	.036	.037	.038	.039	.040	.041	.042	.043	.044
6	6	6	17	20	17	30	45	70	63	68	75	95	95	85	73

.044	.045	.046	.047	.048	.049	.050	.051	.052	.053	.054	.055	.056	.057	.058	.059
.045	.046	.047	.048	.049	.050	.051	.052	.053	.054	.055	.056	.057	.058	.059	.060
55	53	39	28	33	24	20	7	5	5	6	5	3	1	0	1

This table is to be considered in the same way as the first.

The time as given is the total time, or that which elapsed between the delivery of the blow and the falling of the eyelid, and hence included the true reflex time and the time taken by the peripheral mechanism.

It does not seem advisable to reduce these numbers to such as would represent the time actually consumed by the reflex centre, as to do so it would be necessary to subtract, as Exner did, the time taken by the transmission of the nerve impulse and the latent period of the muscle. As a matter of fact nothing accurately is known about

either one of these. Helmholtz* estimated a rate of nerve transmission of 34 m. to the second in man; Schelske† about the same. Oehl‡ gives a speed of sensory transmission of 35 m. in the winter months and 41 in the spring. A. M. Block§ believes he has proved the speed of sensory nerve transmission to be 132-141 m. per second. Which of these speeds is to be accepted? If the slowest, that of Helmholtz, 0.0103 second, must be subtracted from the total time; if the fastest, that of Block, 0.0025 second.

Again, nothing absolutely is known as to the latent period of the orbicularis palpebrarum. In experiments of such delicacy it would not be right to assume the latent period of any given muscle from experiments on another, especially if the one experimented on be from one of the lower animals. Until these problems are settled, it seems best to leave the question as to the exact amount to subtract an open one. Therefore, in all the tables and charts the total times are given.

The results when submitted to inspection showed greater variations than could be explained by the irregularities of the apparatus or the errors of reading. The only alternative, then, was to attribute them to some physiological cause. As it was thought that natural winks occurring very shortly before the blow was struck might influence the reflex time, all cases in which such winks were known to have occurred were noted. Here came in one of the advantages of the apparatus. As has before been mentioned, every time the subject winked, the operator in the other room, by the recording apparatus, was cognizant of it, as every time a wink occurred the pointer of the signal rose. The usual method of operation was as follows: After the adjustments at eye and stimulator had been made (if necessary) after each blow, the experimenter waited, watching the signal. As a rule the trembling of the pointer would indicate that at first the subject's mind was not intent on the work given him to do. The record, therefore,

* *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1850, pp. 71-296, and 1852, p. 199.
† Ibid., 1864.
‡ *Arch. Ital. de Biologie*, vol. 21, p. 401.
§ *Journal de l'Anat. et Physiol.*, vol. xx, pp. 284-290, Paris, 1884.

would not be taken until the steadying of the pointer and the regularly recurring wink showed that the subject was as calm as could be expected. Sometimes a wink would take place after the drum had started to revolve. The curve would show whether or not it was completed before the blow was delivered. If it was, and the reflex wink occurred independently of it, note was made of that fact. A series of twenty-six such cases has been collected, in which a natural wink was completed very shortly before the blow was delivered. Their evidence is negative. Sometimes they are longer and sometimes shorter than the records which immediately precede and follow them. Although the series is somewhat inadequate, it suggests that such closely preceding winks have no effect.

Personality has in a general way some effect. Thus, if the averages of the results obtained in the different sets of experiments upon the different men be arranged in three classes—slow, medium, and fast—it will be found that all the averages of experiments taken under the same conditions from any one person will fall in the same class. Of course no hard and fast line can be drawn between such classes, as they merge insensibly into one another. The following tables give the records of the two men who occupied the extremes. Both the correspondence between the results of experiments on the same man and the differences between the two are well shown.

X.		Y.	
1894.	1895.	4-3-'95.	4-4-'95.
0.0380	0.0349	0.0554	0.0484
0.0396	0.0318	0.0508	0.0496
0.0388	0.0337	0.0489	0.0487
0.0347	0.0333	0.0443	0.0505
0.0370	0.0362	0.0493	0.0503
0.0349	0.0330	0.0462	0.0471
0.0353	0.0323	0.0457	0.0507
0.0392	0.0352	0.0489	0.0557
0.0382	0.0362	0.0445	0.0545
0.0396	0.0351	0.0481	0.0494
0.0369	0.0360	0.0430	0.0452
0.0417	0.0331	0.0494	0.0473
0.0377	0.0372	0.0467	0.0438
0.0398	0.0305	0.0485	0.0419
0.0358	0.0346	0.0481	0.0461

0.0365	0.0329	0.0468	0.0471
0.0360	0.0396	0.0475	0.0488
0.0360	0.0341	0.0499	0.0492
0.0398	0.0345	0.0467	0.0486
0.0383	0.0407	0.0508	0.0505
0.0372	0.0350	0.0482	0.0485
0.0326	0.0345	0.0460	0.0504
0.0356	0.0396	0.0469	0.0516
0.0349	0.0376	0.0480	0.0530
0.0338	0.0343	0.0473	0.0527
0.0386	0.0336	0.0485	25)1.2296
0.0365	0.0375	26)1.2444	0.0491
0.0319	0.0398	0.0482	
28)1.0349	0.0344		
0.0369	0.0329		
	0.0339		
	31)1.0900		
	0.0351		

The correspondence between the two sets on X is the more striking when it is remembered that they were taken a year apart, the first in the spring of 1894 and the second in the spring of 1895.

Sex seemed to have no influence; the differences between the mean times of the women experimented on, as compared with the results from men, being no greater than the differences in time between different men.

The influence of the mind on the reflex time had to be considered. It was suggested that the minds of the subjects be occupied in some definite and constant way in order to see if this would have any effect. To this end, during certain of the experiments (series a of Plates VI-VIII), the subjects were to multiply together or to add two numbers written on the block at which they looked and to remember the results. During others (series c of Plates VI-VIII) a manometer with a rubber bulb attached to one arm was placed before them, and they were required to keep the level of the mercury at a certain height. This it was supposed would occupy the mind and at the same time exercise a certain set of muscles. During still others (series b of Plates VI-VIII) the subject was given a warning a few seconds before the blow that it was about to fall. After a few trials of these methods the character of the curves suggested that the subjects were not always occupying

their minds in the indicated way so completely as might be wished. They were therefore questioned as to the state of their feelings during the experiments. The replies given did not correspond with one another. Some said they were apprehensive during the arithmetical problem, some when the warning was given, while some were not at all apprehensive during any of the time.

The experiments were taken in sets of three series. During one series, which would consist of as many experiments as might be recorded on one drum (from 10-12), the subject would perform addition or multiplication (series a); during another, taken as soon as another drum could be substituted, the warning would be given (series b); and during the third he would be required to press the bulb of the manometer (series c). Lest the subject might grow slightly weary, and thus the time be influenced, the order of taking was changed when more than one set was taken on a single individual.

Experiments were thus taken on nine different persons. On one of these eight different sets were taken within a week, with a view of ascertaining whether or not habit would have any influence. The records would seem to show that it does not (see Plate VIII, A).

When all the experiments were taken and the readings made, the results were plotted in order to get a better idea of them. The resulting curves show nothing regular (see Plates VI and VII). A curve of averages was then made. The curves from the different subjects did not correspond to one another, but those from the same subject in the two cases where more than one set was taken had a general resemblance (Plate VIII, A and B). When the statements as to the subject's feeling of apprehension were applied to these, a little light was thrown on them. It was seen that in those experiments during which apprehension was felt the average time was shorter (see Plate VIII). This fact is suggestive of the Mental Reinforcement of the Knee Jerk.* The fact that squeezing the bulb of the manometer did not have the same effect may be explained by the fact that the muscular effort was a continuous one.

* Lombard, *American Journal of Psychology*, vol. 1.

Conclusions.

I. The mean total time taken from 450 experiments on sixteen subjects was found to be 0.0420 second.

II. Natural winks occurring very shortly before the record was taken had no influence.

III. The reflex time was found to vary in different individuals, the limits of the mean times being 0.0351 and 0.0491 second. For any given individual there was a close correspondence between averages of sets of experiments obtained under similar conditions. In the case of one man this was true of sets of experiments taken a year apart. Sex has no influence.

IV. Apprehension seems to shorten the time.

Description of Plates IV-VIII.

Plate IV.

Fig. 1.—Apparatus used in determining the time of reflex winking. *a*, platinum foil on eyelid; *b*, platinum wire which touches the foil. It is mounted on the movable bar, *f*, which is fastened to frame and connected through a wire to the binding post, *g*. *c*, the breech-block of the stimulator; *d*, wires bearing current to electro-magnet of stimulator; *e*, wires bearing current to breech-block. In taking the photograph the camera was placed on a level below that of the head in order to get a better view of the apparatus. As a result, the head appears thrown farther back than was really the case.

Fig. 2.—Stimulator used to excite reflex winking. *a*, soft iron head of spring hammer; *b*, screw which regulates tension of spring of hammer; *c*, hard rubber plunger passing through breech-block; *d*, brass breech-block; *e*, brass pin running through plunger and connected by means of the time wire with the binding post, *g*; *f*, screw, by loosening which the breech-block may be lowered so as to increase length of blow of hammer; *h*, *i*, binding posts connected with coil of electro-magnet; *k*, pole of electro-magnet which holds back the hammer. The recording current runs from *f* to *d* into *e* and along wire to *g*, thence to platinum foil on eyelid.

Plate V.

Recording Drum and Attachments.—*A*, Desprez signals; *b*, double wire bearing current flowing through eye connections and stimulator; *c*, *d*, wires from tuning fork; *e*, key through which current to electro-magnet of stimulator runs; *g*, *f*, wires bearing this current.

Plates VI-VIII.

See explanation in text, pp. 45 and 46.

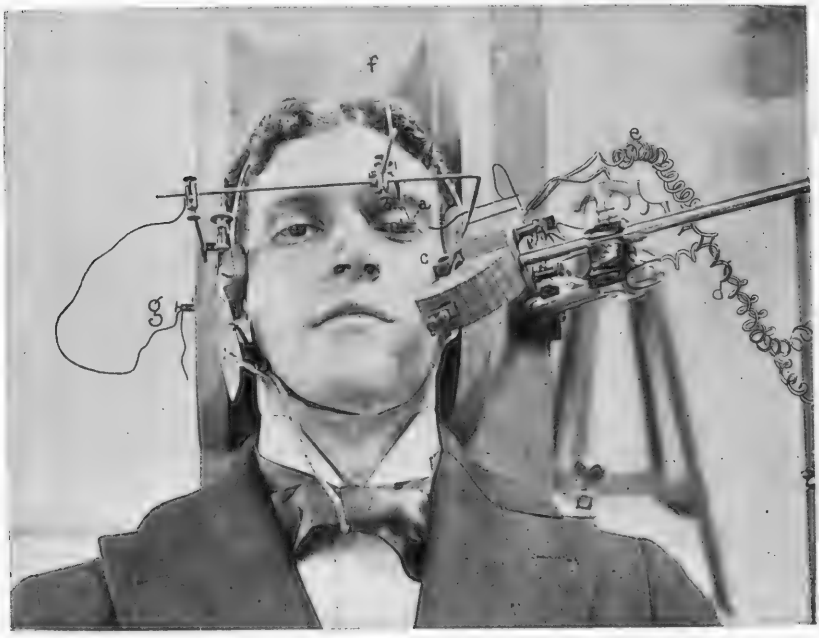


FIG. 1.

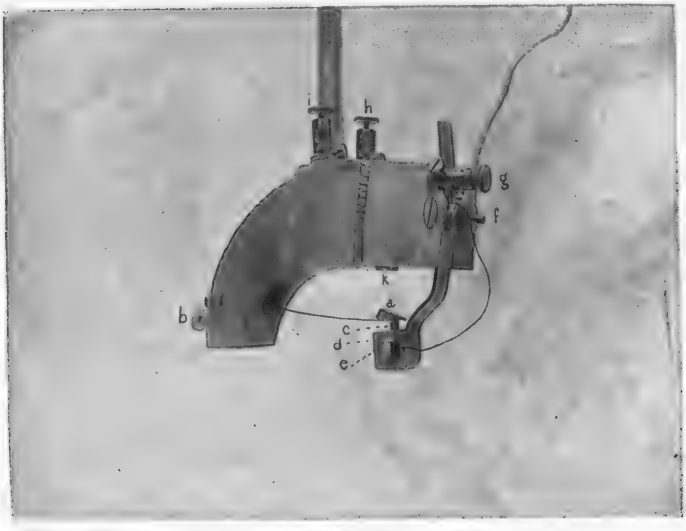
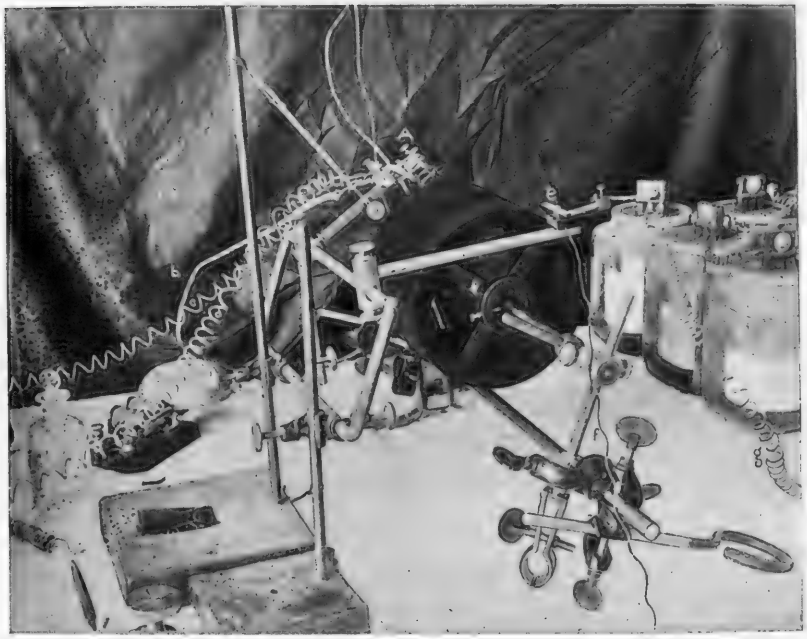


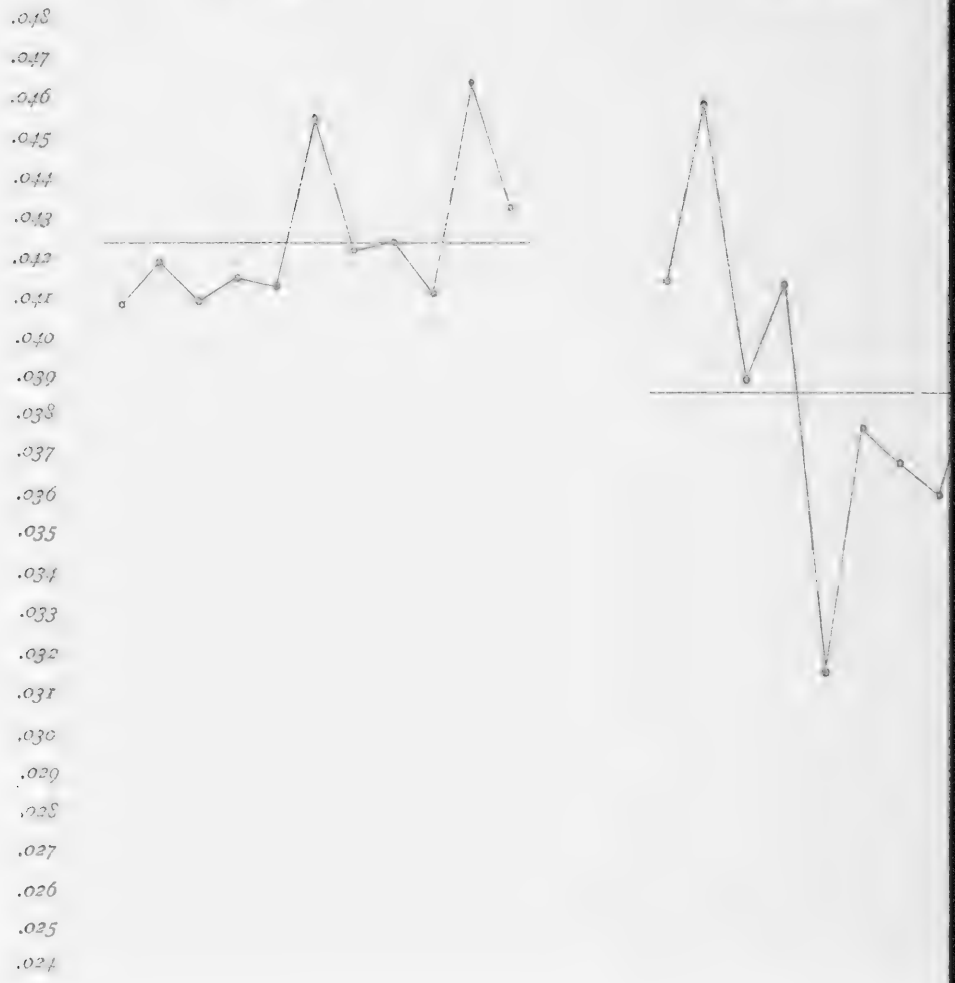
FIG. 2.



Experiments on A.

Series a.
Adding.

Series b.
Warning given.



Experiments on A.

Series b.

Warning given.

Series .c.
Pressing Manometer Bulb.

Experiments on A.

Series a.
Adding.

Series b.
Warning given.

Experiments on A.

Series I.

Warning glass.

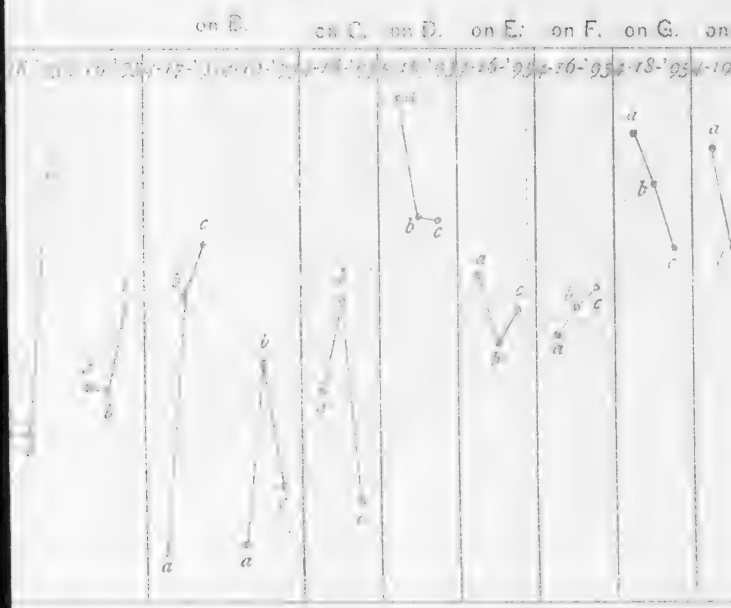
Series c.

Table of Averages.

a = Adding. b = Warning given.

Table of Averages.

b = Warning given. c = Pressing M



[illegible] but none at all when [illegible]

[illegible] warning [illegible]

[illegible] both when the [illegible]

[illegible]

[illegible] not looked out for blow during (b) and (c). He could not say during

[illegible] could not say during which she worried most.

[illegible]

nometer Bulb.

AN EXPERIMENTAL INVESTIGATION OF SOME OF THE CONDITIONS INFLUENCING THE SECRETION AND COMPOSITION OF HUMAN BILE.

By FRANZ PFAFF, M. D., Ph. D.,

AND

ALFRED W. BALCH, Student of Medicine.

(*From the Pharmacological Laboratory of the Harvard Medical School.*)

Plate IX.

In this article we shall relate the results of an experimental investigation of some of the conditions influencing the secretion and composition of bile. The subject of the experiment was a female patient at the Massachusetts General Hospital, and we desire to express our great indebtedness to Dr. H. H. A. Beach, who most kindly placed the case at our disposal and who helped us in every way possible to carry on the investigation to the desired end. We have also to thank Dr. Beach's house surgeon, Dr. John C. Pegram, Jr., who took a great deal of care and trouble in superintending the general management of the case at the hospital and in collecting the bile. The arrangement for doing this, which was devised by him, answered its purpose most satisfactorily.

The following report of the history of the case was kindly given us by Dr. Pegram:

On December 10th, 1894, Mrs. M. entered the hospital in the service of Dr. F. C. Shattuck, with the following history: Age 38. Family history negative. Previous history: measles and scarlatina as a child. Married ten years, five children, the youngest nine weeks old. Had acute rheumatism two years ago, for which she was treated in this hospital. Never any rheumatism before or since. Pregnancy uneventful until four months ago. Had during the last three months three chills, which were called malarial. Fever and sweating followed chills. Weighed 160 lbs.

before onset of present trouble. Four months ago had a pain beneath the right costal border. There were nausea and retching with the pain, but no vomiting. No chill nor fever, but profuse sweating. Pain lasted three hours. Jaundice noticed two days later, which persisted in a varying degree. Color deepened after each attack of pain, and faded at intervals until the last attack, after which it remained unchanged. Eleven weeks ago a second attack of pain, four weeks ago a third, and two weeks ago the last and most severe attack. Urine for several days after each attack was dark and then would become normal in color. Stools noticed to be clay-colored on only one occasion; not offensive. Bowels moved daily.

Physical examination.—Well developed, thin woman, marked yellowness of skin and conjunctivæ. Lungs and heart negative. Marks of scratching on back and arms. Liver was felt two inches below right costal border, and what seemed like an enlarged gall bladder was felt projecting below. Urine at this time: bile color, acid, slight trace of albumin, renal cells of normal appearance, some bile stained. Some abnormal blood. A few hyaline and granular casts of small diameter. Fæces: hard, some well colored, others clay-colored.

Operation was advised but was refused. She was treated then with euonymin gr. iii twice a day. Color faded some three days later. One stool was dark and another light. Tumor was as described above, but no pain. December 28th, 18 days after entrance, she was discharged. Urine then showed only a faint trace of bile.

On April 4th, 1895, the patient again entered the hospital, this time in the service of Dr. M. H. Richardson, giving the following history: Since leaving the hospital her general health was good except for three attacks of pain, followed by jaundice, similar to previous attacks. Physical examination at this time showed a slight jaundice. Just below right costal border and about in the anterior axillary line was felt a tumor the size of a hen's egg, soft and movable, dull on percussion. Liver apparently not increased in size. Patient had an attack of pain on day of entrance and another the following day. On the day of entrance stools clay-colored. April 8th, cholecystotomy by Dr. M. H. Richardson. Seven large gall stones removed from the cystic duct. None were felt in the common duct. By April 20th patient was having movements of good color, jaundice practically gone. By April 23rd the wound healed, and on this day she was discharged.

On November 4th, 1895, patient re-entered the hospital, this time in the service of Dr. H. H. A. Beach, and gave this history: Shortly after

last discharge from the hospital she had an attack of colic, which passed off. Four or five weeks later she had a second attack, followed by slight jaundice. At this time a small abscess formed in the cicatrix from operation, and, according to the patient's account, this broke and discharged bile for three or four weeks and then healed. She was comparatively well during the next three months. Five weeks ago she had a third attack, and after that had three more. Examination at entrance showed the patient to be very much jaundiced, conjunctivæ yellow. Distinct mass felt in region of gall bladder. Urine high colored with bile pigment. Fæces clay-colored.

November 8th, operation performed by Dr. H. H. A. Beach. A rapid dissection through the old cicatricial tissue soon disclosed the much distended gall bladder, which was opened, giving vent to nearly a pint of thin and dark green bile. A careful search for impacted calculi or other obstructions was now made with probes entered at the cystic duct. No calculi were found. The opening in the gall bladder was now closed temporarily and a careful dissection made below it into the general peritoneal cavity. An investigation of the relations of the common duct was then made to determine whether the canal was obstructed, the exploration extending toward the left side as far as the aorta. The probe reinserted could be felt from the outside of the gall bladder extending to a mass that seemed to include the head of the pancreas, which appeared to be enlarged and thickened as though it were the seat of old inflammatory processes or of some new formation. The wound was then closed, with the exception of the gall bladder opening, which was drained by a gauze wick. The wound granulated, and by December 22nd there was left only a sinus leading into the gall bladder. A small rubber drainage tube was inserted into the sinus, and through it the bile drained into a bottle which the patient carried at her side, and she was allowed to walk about the ward. Jaundice disappeared and the patient felt perfectly well and began to gain some in weight. Stools were clay-colored, large and very offensive. The patient avoided fats in her diet, as they caused nausea. From December 22nd, 1895, to January 10th, 1896, the patient excreted through the tube an average daily amount of 15 ounces of bile, an unestimated amount being lost in the dressing.

A careful examination of the depth and calibre of the fistula at this time revealed what one might expect, namely, that, although the opening of the fistula in the skin would just admit a glass tube the diameter of an ordinary lead pencil, beneath the skin the fistula was of a greater diameter. To prevent leakage of bile, in order to keep an accurate

record of the amount of bile secreted, it was simply necessary then to get a cannula with a bulbed end into the fistula, the bulb of course to be larger than the opening. By taking a number twelve soft rubber catheter, invaginating it into a piece of rubber tubing, and then reflecting the rubber tubing on itself for two inches and then cutting off the pointed end of the catheter, we had a pliable cannula bulbed at one end. This bulb was larger than the fistulous opening in the skin, but by dilating the opening a little and exerting a little force the cannula slipped into the sinus. In twelve hours the scar tissue, which was stretched in dilating, contracted sufficiently to hold the drainage tube in place and prevent the escape of bile, save through the lumen of the catheter, which was then connected with a glass tubing which ran over the edge of the bed and emptied into a graduated glass jar. The introduction of the cannula into the wound caused no particular change there, nor did it cause any irritation. Patient was still wearing her cannula when discharged from the hospital on April 18th, 1896.

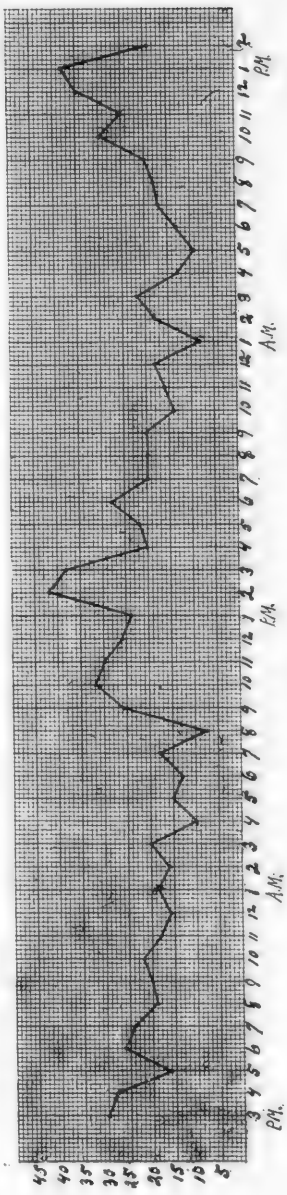
We intended to study the action of some of the supposed cholagogues. Before entering on the real subject of our investigation we had to study first the normal flow of bile in our patient and the normally occurring variations. The patient was taking the ordinary hospital diet,* consisting of breakfast, dinner and supper.

As we wished first to determine accurately the hourly quantities of bile discharged through the fistula, the patient was kept in bed for two consecutive days, and the amount of bile collected during each hour was measured. On the third day the patient was up and walked about the ward. On this day the total quantity of bile passed during 24 hours was collected and analyzed.

The chart on page 53 represents the hourly flow of bile during 48 consecutive hours.

* This diet was made up for breakfast as follows: oatmeal, wheat or ceraline, baked potato, bread, butter, toast, tea, coffee. For dinner: potato and one other vegetable, pudding or ice cream, bread, butter, toast, tea. For supper: cream toast, cooked fruit, cereal food or blanc mange, bread, butter, toast, tea, cocoa. In addition, soup every day for dinner, roast beef four times a week, lamb stew once, baked fish once, and roast lamb once. In addition there was given for breakfast on Monday, scrambled eggs; Tuesday, fresh fish in cream sauce; Wednesday and Saturday, hash; Thursday, broiled minced beef or salt fish and cream; Friday, salt fish and cream or scrambled eggs; Sunday, baked beans or steak.

We began to collect the bile at 2 P. M. on the 12th of January, 1895. At 1 o'clock the patient had her dinner, at 6 supper, and at 7 o'clock the following morning breakfast. On the abscissa of the curve are marked the hours of the day, and on the ordinate the quantities in cc. of bile collected during each hour.



At a glance we see that the hourly quantities of bile secreted vary very much, the lowest amount being 8 cc. and the highest 42 cc. On one day the lowest quantity of bile was collected in the morning from 7 to 8, and on the other day from 12 to 1 o'clock A. M. The largest quantity secreted fell on one day between 1 and 2 o'clock P. M.; on the other day from 12 to 1 o'clock P. M., that is to say, one day directly after dinner and the other day directly before dinner.

The total quantity collected on the third day, when the patient was up and walking about, was 525 cc., whereas the amounts for the first two days were 517 and 501 cc. of bile per 24 hours. The daily total quantity of bile secreted by the patient seemed therefore to be rather constant.

Before we began our experiments a chart of the daily amount of bile discharged through the fistula had been kept for a few weeks—showing an average quantity of 15 ounces of bile per day. But at this time a certain

quantity was absorbed by the dressings, and therefore the total quantity collected must have been smaller than that found by us.

The specific gravity of the different samples of bile, collected during these three days, varied from 1008 to 1010. The color was yellowish brown. A small trace of albumin was present. The percentage of solids varied between 1.6 and 1.7 per cent, and the ash between 0.8 and 0.9 per cent.

To compare our results of the flow of bile in a case of biliary fistula, we will review the observations made upon similar patients by other observers.

Monro* observed in a case of abscess of the liver communicating with the gall bladder and lung, the flow of bile, which was discharged by coughing. The quantity expectorated, being mixed with mucus and saliva, could not be measured with great accuracy. The whole quantity in 24 hours was from 10 to 15 ounces. The quantity of bile discharged differed at different times. It was always greater after meals, and especially for an hour or two after dinner.

Charles Robin† quotes a case of Taconi in which an old female patient secreted through a biliary fistula 500 grammes of bile in 24 hours. The cause of the biliary fistula is not given.

Johannes Ranke‡ observed a male patient with a fistulous opening between the liver and the lung. Sometimes the total quantity of bile was discharged by coughing, at other times the bile found its way, apparently in total quantity, into the intestine. Five determinations of the quantity of bile secreted during 24 hours were made at a time when the fæces did not show any trace of biliary constituents. The bile was of course mixed with the bronchial secretion and saliva. As soon as the bile ceased to be coughed up, Ranke determined the amount of bronchial secretion which was expectorated the next 24 hours and deducted this amount from the quantity of mixed biliary and bronchial secretion. The average of bile secreted during the five days of observation was thus calculated to be 636 cc. per day. The minimum on one day was 405 cc.,

* Quoted in The Cyclopædia of Anatomy and Physiology. Edited by R. B. Todd. London, 1839-1847, p. 180.

† Leçons sur les humeurs normales et morbides du corps de l'homme. Paris, 1867.

‡ Die Blutvertheilung und der Thätigkeitswechsel der Organe. Leipzig. 1871.

and the maximum on another day 922 cc., a difference of over 100 per cent.

M. Noel* published a quantitative analysis of a sample of bile collected from a patient with a biliary fistula. He does not give any cause for the biliary fistula, and states only that the quantity of bile discharged during 24 hours was about 900 grammes on one occasion. The specific gravity was 1009.7.

Von Wittich† observed for a very short time a patient in whom a biliary fistula had formed spontaneously on account of gall stones in the cystic duct. Wittich collected in four hours during the daytime 88 cc. of bile, and during ten hours of the night 224 cc. From these two observations he calculated the amount of bile secreted per hour as 22.2, and per day as 532.8 cc. He made no analyses of solids or ash.

In 1873 H. Westphalen‡ observed the flow of bile in a patient who probably had a liver abscess, causing perforation into the right pleural sac, with subsequent discharge of the bile through the bronchi. Thoracocentesis being performed, the bronchial fistula closed, and for about three weeks the bile was discharged through the opening in the thorax. The bile was measured every four hours, day and night. The amount discharged for four hours did not vary much during the day, whereas the quantity collected from 2-6 A. M. was invariably the smallest. The bile passed during the night was more concentrated than during the day. The total amount of bile discharged during 24 hours amounted on the average to 498.85 grammes of 1010.4 sp. g., with 2.25 per cent solids.

Charles Murchison§ saw a case of biliary fistula operated on on account of biliary concretions. He made no analyses, and merely says "the fluid was discharged from the opening at a rate of from 1 to 2 ounces in one hour, sometimes more and sometimes less. It was usually increased after a meal."

In 1884 Gerald F. Yeo and E. F. Herroun¶ published their observations made on a patient with cancer of the common bile duct. The average amount of bile secreted per 24 hours was only 374.5 cc. The total amount of solids was in the mean 1.35 per cent, and the sp g. 1008.

* Recherches sur la bile prise sur un malade atteint de fistule biliaire. *Journal de Pharmacie et de Chimie*, III, vol. xxxxi, p. 354, Paris.

† Zur Physiologie der menschlichen Galle. Pflüger's *Archiv*, vi (1872), p. 181.

‡ Ein Fall von Gallenfistel. *Deutsches Arch. f. klin. Medicin*, xi, p. 588, 1873.

§ Clinical Lectures on Diseases of the Liver. Second edition, New York, 1877.

¶ A Note on the Composition of Human Bile obtained from a Fistula. *The Journal of Physiology*, vol. v, pp. 116-123.

"No difference in rapidity of secretion could be detected at any time, and no increase was noticed after the small meals of which the patient partook; the amount produced at night-time was the same as that secreted during an equal period in the day."

S. Monckton Copeman and W. B. Winston* published in 1889 their observations made on a female patient suffering from biliary colic due to gall stones. A biliary fistula was established, and the total quantity of bile secreted during 24 hours was measured every day at 10 A. M. During more than a month the bile was collected every day. The average amount of bile secreted during 24 hours was 779.6 cc. The amount of solids was estimated on four different occasions. On one the amount was 1.42 per cent, or total 10.86 grammes solids per 24 hours.

On different days Copeman and Winston made observations on their patient to determine the hourly variations in the flow of bile and the relation of the variations to the meals taken by the patient. These observations always extended over 24 hours. The mean of all these variations is given in a curve. The patient had her meals as follows: Breakfast, 5 A. M.; lunch, 10 A. M.; dinner, 1 P. M.; tea, 4.30 P. M.; and supper, 7.30 P. M. "The secretion was least at 5 A. M., when some hours had elapsed since the last meal, and greatest at 12 noon, the amount ranging between the wide limits of 14.5 cc. in an hour and 54.5 cc. in an equal time. Breakfast time, when the rate of the flow was at its lowest ebb, is followed by an immediate rise for the next hour, with a secondary one two hours later. The extensive rise about noon is curious, as the 10 o'clock lunch is not by any means a large meal, while there is no such elevation after dinner, the principal meal of the day."

In 1890 A. W. Mayo Robson† published his observation made also on a female patient in whom he had established a biliary fistula on account of obstruction in the common bile duct. The bile escaped through the fistula for 15 months. Later cholecystenterostomy was performed and communication with the duodenum thus re-established; the patient making after both operations good recovery. The quantity of bile collected in 24 hours on various dates during eight months of observation varied from 39.5 ounces to 25.9 ounces, and averaged nearly 30 ounces. More bile was invariably secreted during the day than at night, the difference at times being as much as 5 ounces, at others not more than 3 drams. "More solids are contained in the bile by night than by day.

* Observations on Human Bile obtained from a Case of Biliary Fistula. *The Journal of Physiology*, vol. x, pp. 213-231.

† Observations on the Secretion of Bile in a Case of Biliary Fistula. *Proceedings of the Royal Society*, vol. xlvii, p. 499.

The quantity of bile discharged is apparently not much influenced by the ingestion of food. The reception of food into the stomach is generally contemporaneous with a marked decline in the flow of bile, lasting about two hours." On page 504 a curve is published representing the hourly secretion of bile during 24 consecutive hours. The smallest quantities of bile were discharged in the early morning hours; the highest amount on one day at 5 P. M. and the other day 9 A. M.

D. Noël Paton and John M. Balfour* made in 1891 a series of observations also on a female patient suffering from cholelithiasis. After a biliary fistula had been established they determined that the total quantity of bile excreted in 24 hours was on an average 638 cc. The specific gravity varied between 1005.5 and 1008.2. The solids amounted to 1.31 per cent, and the average total weight of the bile solids in 24 hours was 8.38 grammes. But the patient at the time of the investigation was not normal, as shown by abnormal temperature, etc.

A year later Noël Paton† made on the same patient new observations, during two days only, but the patient was now in perfect health. The biliary fistula had remained open during the whole time, the total quantity of bile being discharged through the fistula. The total quantity of bile secreted during 24 hours was (mean of the two days' observation) 590 cc., with 2.3 per cent of solids, the total bile solids excreted per 24 hours amounting to 13.6 grammes.

Olof Hammarsten‡ observed the flow of bile in seven patients with biliary fistula. In each of his seven cases some of the bile found its way into the intestine. The total quantity of bile secreted per day was therefore not entirely discharged through the fistula and could not be measured. The percentage of solids in the different samples collected by Hammarsten varied from 1.63 per cent to 3.53 per cent. In most of his cases this percentage was higher than that found by other observers. This fact may be explained by the circumstance that bile really found its way into the intestine. Hammarsten's samples of bile are therefore to be regarded as the sum of the fresh bile formed daily

* On the Composition, Flow and Physiological Action of Bile in Man. Reprinted from vol. iii of Laboratory Reports issued by the Royal College of Physicians, Edinburgh, 1891. Quoted from Gamgee's Physiological Chemistry, 1893.

† D. Noël Paton, Further Observations on the Composition and Flow of the Bile in Man. Laboratory Reports of the Royal College of Physicians, Edinburgh, vol. iv, 1892. Quoted from Gamgee's Physiological Chemistry, 1893.

‡ Zur Kenntniss der Lebergalle des Menschen. Maly's *Jahres-Bericht der Thier-Chemie*, 1894, p. 331.

and a part of reabsorbed bile. That part of the bile which entered the intestine had of course its influence upon the digestive processes taking place there, and thus may have also influenced the amount of solids in the biliary secretion.

In the beginning of 1896 Edington* published observations made on a patient with a biliary fistula. He collected from 285.5 to 70.5 cc. bile per day. But he could not collect the total amount of bile secreted during 24 hours, as some of it always found its way into the duodenum, the obstruction to the outflow being incomplete.

Of all these different observations on the flow of bile in human subjects, there are only three which may really be compared with our case. The others are either too incomplete or were made on subjects in abnormal conditions, so that the results obtained are not reliable. Taconi's, von Wittich's, Noël's and Murchison's cases are too incomplete to allow any comparisons. In Ranke's, Westphalen's, Monro's, Yeo and Herroun's cases the patients were in abnormal conditions, so that the results obtained are not reliable. In Hammarsten's seven cases and in Edington's patient the bile entered partly into the duodenum, so that the total quantity could not be ascertained. The remaining cases of Copeman and Winston, Mayo Robson and Noël Paton's second observation gave results obtained on patients whose condition, with the exception of the biliary fistula, could be fairly well regarded as normal, and which can therefore be compared with the data in our case. In all four cases the patient was a female. Comparison of the quantities of bile and bile solids secreted during 24 hours in these different cases will be seen in the following table.

TABLE I.

	Copeman and Winston.	M. Robson.	Noël Paton.	Our patient.
Weight in K grs.	43.7	53	? 73	51.4
Bile secreted in cc.	779.6	849.0	590.0	514.3
Bile solids in grms.	11.09	15.28	13.596	8.57
Bile secreted per K of body in cc...	17.08	16.0	8.08	10.0
Bile solids secreted per K of body..	0.25	0.28	0.186	0.167

* The Bile Salts in their Relation to the Secretion of Urea, etc. *Journal of Anatomy and Physiology*, 1896.

The total quantity of bile secreted during 24 hours was smallest in the case of our patient; the same is true of the total amount of bile solids. For completeness we give also in the table the bile and bile solids secreted in proportion to one kilo body weight of our patient. In most of the publications on this subject such comparisons are made by the different authors. A. Gamgee* seems to lay particular stress on this point, and the figures in the above table are copied from his book. But body weight and bile secretion do not stand in definite relations, and comparisons cannot well be made. It is an often observed fact that small animals of the same species frequently furnish absolutely larger quantities of bile than larger animals of the same kind. P. Spiro,† and before him Ritter,‡ showed that bile secretion and body weight do not stand in any relation to each other. We shall see later that our patient, in spite of a considerable gain in weight, did not secrete a proportionately larger quantity of bile per day.

As to the hourly secretion of bile we can well compare the curves given by Mayo Robson, and by Copeman and Winston, with ours. In their cases and in our observation the first things to notice are the enormous variations in the hourly flow of bile and the lack of all regularity. The greatest amount of bile secreted may occur at any time of the day, and cannot be placed in definite relation to any meal. Broadly speaking, we can deduce from our combined observations only the fact that the flow of bile increases during the day, after taking the different meals, and that it decreases again during the night. The hourly quantities of bile secreted are very irregular. The smallest amount of bile secreted is, as a rule, in the early morning hours, before any food is taken. The night bile is more concentrated than the day bile.

This is in contradiction to the observations of A. Kunkel§ made on dogs. He found that the bile secreted during the day was more con-

* Text-book of the Physiological Chemistry of the Animal Body. By Arthur Gamgee. London, 1893.

† Ueber die Gallenbildung beim Hunde. *Arch. f. Physiologie*, 1880, Supplement Band, p. 62.

‡ Marburg, 1862. Quoted from Spiro's paper.

§ D. A. Kunkel, Pflüger's *Archiv*, xv, p. 353 (1877).

centrated than during the night, whereas urine was more dilute during the day than at night.

Our observation that the night bile is generally, if not invariably, more concentrated is corroborated by the observations of Mayo Robson and Westphalen on their patients, who also found the night bile more concentrated.

As the hourly quantities of bile vary so enormously, an experimental study of the action of supposed cholagogues, based on observations of the hourly flow, would be positively illusory. Normally occurring variations would be held for the supposed effect of drugs, and conclusions drawn from such results would be entirely without foundation. As we have seen, the flow of bile did not vary very much in the total quantities discharged during 24 hours, the amounts being between 525 and 501 cc. in an observation of three consecutive days. Comparisons of the variations of the daily quantities of bile secreted could therefore give positive results as to the real value of supposed cholagogues. But in order to minimize here the possible errors in excluding normally occurring variations even in the daily flow, we concluded to compare the amount of bile discharged during consecutive normal days when the patient was taking no medicine, with another period of days when the patient was under the influence of so-called cholagogues. Such a method of experimenting had to be extended over a rather long period to show results for each substance with which experiments were made. But our aim was not to try many substances for their supposed action, but rather to study accurately only a few drugs, in order to get, if possible, a definite knowledge of their action.

Many experimental investigations of the action of supposed cholagogues on animals have been undertaken, and this shows the great importance given to this subject. Unfortunately the results obtained were not in direct proportion to the laborious work which those investigations demanded. The whole chapter of cholagogues is really a chapter of contradictions, and only in the last few years has clearness been gradually brought into the experimental part of this important branch of therapeutics. Former experimental investigations in this

line, especially on animals, which gave such contradictory results, have often been undertaken under conditions which are open to severe criticism.

The principal reason why the experiments of the different investigators gave such different results was an erroneous idea as to the normal flow of bile. Most of the experimenters labored under the false impression that the flow of bile was not only continuous, but fairly regular, so that experiments extending over hours would give positive results. In such experiments, variations in the flow of bile, observed during very short time units—quarter-hours, or at the most a few hours—were compared, and the differences found were attributed to the supposed action of the drugs. Whereas in fact these differences in the flow of bile were mostly normally occurring variations! Besides this fundamental error in the experiment, the investigators often used drugs in quantities which would have allowed one to study the toxicological action of the substances employed, but scarcely the pharmacology of these drugs. As an example we may mention a study of the influence of croton oil as a cholagogue where doses of 30 drops,* and even more,† have been given to dogs. The autopsies showed clearly the ravages croton oil made in the intestinal tract of such animals.‡

We are especially indebted to Baldi and to E. Stadelmann and his pupils for having placed the experimental investigation of cholagogues on animals on a sound basis. Before stating their results we will mention the different results of previous experimenters.

The first experimental investigation of cholagogues was performed by Nasse.§ He experimented on a dog with a permanent biliary fistula. First he studied the influence of different food-stuffs upon the flow of

* William Rutherford, On the Physiological Actions of Drugs on the Secretion of Bile. Transactions of the Royal Society of Edinburgh, vol. xxix, p. 146. R. gave these large doses in imitation of Roehrig's large doses.

† Roehrig, Experimentelle Untersuchungen über die Physiologie der Gallenabsonderung. Stricker's *Jahrbücher*, 1873.

‡ Rutherford, loc. cit.

§ Commentatio de bilis quotidie a cane secreta copia et indole. Abstracted in Canstatt's *Jahresbericht*, 1852, p. 155.

bile. Later he mixed with the food on one occasion 1 gramme of calomel. H. Nasse concludes from his results that calomel increases the total quantity of bile secreted, but that it diminishes its solids.

Koelliker and Mueller* experimented on a dog with a permanent biliary fistula. They studied the effect of calomel, and to some extent also of aloes, the observations being made on different days during very short units of time. Calomel during one experiment increased the secretion of bile slightly, and in further experiments it seemed to diminish the flow of bile. Aloes did not seem to have any action whatever.

Mosler† experimented on dogs with permanent biliary fistulæ. As the title of his publication shows, he did not make a special study of cholagogues. Only with one of the drugs employed, namely, calomel, did he mention that he could not find any noticeable increase in the flow of bile, as practitioners seem to believe.

G. Scott‡ made his observations on a dog with a permanent biliary fistula, usually during 24 hours, sometimes for a longer period, and compared the variations in the amount of bile secreted during time units of 24 hours. He came to the conclusion that calomel in large doses produced "a diminution in the amount of fluid bile and bile solids."

The subject of cholagogues was further investigated experimentally by a committee of English physicians, of which Hughes Bennett was chairman.§ The result of this investigation was that mercuric chloride, calomel and pil. hydrargyri did not increase the flow of bile. Podophyllin and taraxacum have also no cholagogue action, according to the same authors. Bennett and his collaborators experimented on dogs with permanent biliary fistulæ.

The next investigation was made by A. Roehrig.¶ He experimented on curarized dogs and rabbits. He counted the drops of bile flowing from the cannula inserted into the opened gall bladder. The time units were measured by a metronome. Roehrig came to the conclusion that croton oil, extr. colocynthidis, sapo jalapinus and aloes acted as powerful

* Bericht über physiologische Versuche. *Vrhdlg. der Würzburger physikalisch-medicinischen Gesellschaft*, Bd. v, 1855.

† Untersuchungen über den Uebergang von Stoffen aus dem Blute in die Galle. Virchow's *Archiv*, Bd. xiii, p. 29, 1858.

‡ On the Influence of Mercurial Preparations on the Secretion of Bile. Beale's *Archives of Medicine*, vol. i, p. 209.

§ *British Association Reports*, 1868.

¶ Abstracted in Virchow u. Hirsch's *Jahresbericht*, 1873, i, p. 143, and Stricker's *Jahrbücher*, 1873.

cholagogues before the aperient effect of these drugs became manifest. Rheum, senna, magnesium sulphate, calomel, and croton oil are, according to this author, less powerful cholagogues.

In 1879 Rutherford* published his experimental investigation of cholagogues. He had as collaborators W. Vignal and W. T. Dodds. These investigators experimented on fasting curarized dogs with temporary fistulæ. They tried a great number of drugs, and came to the conclusion that mercuric chloride, sodium phosphate, jalap, aloes, sodium salicylate, etc., are powerful cholagogues. Calomel, according to their investigation, acts only as an intestinal stimulant, not stimulating the liver. The duration of each experiment was about 6 to 8 hours. Before entering into the study of cholagogues, Rutherford and his collaborators observed first the normal flow of bile in curarized dogs. Two of the published curves show clearly the irregular flow of bile in such animals, and ought to have cautioned the experimenters as to the propriety of drawing conclusions from small variations in the flow of bile observed under the influence of drugs during small time units.

Siegfried Rosenberg† studied the effect of supposed cholagogues on fasting dogs with permanent biliary fistulæ. He concluded that olive oil and fats are the most powerful cholagogues. He studied further the action of salicylate of soda, Durand's mixture (ol. terebinth plus ether), and of Carlsbad salt. The first of these drugs is a cholagogue, according to Rosenberg. Durand's mixture gave no positive results, and Carlsbad salt negative results. Rosenberg published protocols, which, according to him, warranted the conclusions he drew. But we fail to deduce such conclusions from his published results. The only one we can come to is that the flow of bile, even in a fasting animal, is very irregular. Rosenberg often observed about 100 per cent difference in the quantities of bile secreted from one hour to another, while the animal was only fastened to the table and was given no drug whatever. The amount of bile secreted, after olive oil or after food, showed little difference. Rosenberg's unwarranted conclusions found their way into practical medicine, and even in our days patients are drugged with doses of olive oil *ad nauseam*, on the supposition that the oil is a powerful cholagogue.

Attilio Battistini‡ experimented on dogs with a Bernard's fistula. He does not give in detail any results of his experiments with santonin,

* Loc. cit.

† Ueber die cholagoge Wirkung des Olivenöls im Vergleich anderer cholag. Mittel. Pflüger's *Archiv*, 1890, Bd. 46, p. 334.

‡ Einfluss des Santonins auf die Gallenausscheidung. Moleschott's *Untersuchungen*, xiii, p. 414 (1888).

but only tabulates them grossly, so that a verification of his conclusion that santonin is probably the best cholagogue existing is not possible.

T. L. Prévost and Paul Binet* studied a long list of supposed cholagogues and the elimination of medicaments by the biliary secretion. They divided the substances experimented on into (1) cholagogues like urea, terpinol, chlorate of potash, salol, etc.; (2) substances of doubtful properties: Carlsbad salt, aloes, rhubarb, etc.; (3) substances diminishing the flow of bile, such as calomel, atropin, etc. Fats had, according to these authors, no influence upon the secretion of bile. Bile itself is, according to them, "surely the most powerful cholagogue." The experiments were made chiefly on two dogs.

D. Baldi† made his observations on dogs with permanent biliary fistulæ. In his experiments the time of observation varied from 6 hours to 24 and more. Baldi showed from the observations on normal dogs, that is to say dogs that are not under the influence of drugs, that the hourly secretion of bile is very irregular, and that the influence of food upon the secretion is also very irregular. As supposed cholagogues, he tried podophyllin, rhubarb, jalap, sodium phosphate, pilocarpine and Carlsbad water. He concludes that not a single one of these substances increased the amount of bile secreted. He further tried the influence of ox gall on his dogs, giving this drug either *per os*, once 190 ccm., or in small quantities intravenously. After ox gall he always found a decided increase in the secretion of bile.

In late years very carefully conducted investigations of cholagogues have been specially performed on dogs by E. Stadelmann and his pupils, Nissen, Mandelstamm, Mueller, Loewenton, and Glass. Stadelmann‡ summarizes the results of these different observations, showing that with the exception of bile itself, and possibly salicylate of soda, there are no substances which act as cholagogues. He insists that it is absolutely incorrect and faulty, on account of the irregular flow, to draw comparative conclusions as to the flow of bile from observations extending over short periods of time.

A. Gamgee§ does not think that Roehrig's and Rutherford's experiments must be "necessarily" incorrect because other observers have found by other methods different results. He advises that the whole

* Recherches expérimentales relatives à l'action des médicaments sur la sécrétion biliaire et à leur élimination par cette sécrétion. *Compt. rend.*, cvi.

† Recherches expérimentales sur la marche de la sécrétion biliaire. *Arch. ital. de Biologie*, tome iii, p. 389.

‡ *Therapeutische Monatshefte*, 1891, p. 511.

§ Text-book of Physiological Chemistry. By A. Gamgee, p. 347.

chapter of cholagogues be investigated anew with the aid of Schiff's amphobolic biliary fistula. We do not think this is necessary, as Roehrig's and Rutherford's conclusions are based on faulty suppositions, and therefore should not be sustained any longer.

Beside the experimental investigations of cholagogues carried on on dogs and other animals, a few experiments have been made on human subjects with biliary fistulæ.

In Westphalen's* case the effect of large quantities of water, of calomel, and of quinine on the biliary secretion was tried. Large quantities of water did not increase the flow of bile, nor did its quality become altered, whereas the urine of the patient doubled in quantity and its specific gravity diminished. The results agree perfectly with the results of Kunkel,† Stadelmann and other experimenters upon animals. Calomel given in 1.3 grammes did not increase the amount of bile secreted by Westphalen's patient. Quinine given in 2-gramme doses could not be detected in the bile, nor did it affect its flow apparently.

Copeman and Winston‡ gave their patients pills made from purified ox gall, in doses of ten grains three times a day. The first two days there was an increase in the daily amount of bile excreted. But in spite of the patient continuing to take these pills, the amount of bile daily secreted fell after three days to about the same as before. The pills were discontinued after a fortnight without causing thereby any noticeable lessening of the flow. To these results we shall refer again.

Mayo Robson§ tried on his patient, as supposed cholagogues, calomel, rhubarb, podophyllin, carbonate of soda, iridin, turpentine and benzoate of soda. He comes to the conclusion that "the supposed cholagogues investigated seem rather to diminish than increase the amount of bile excreted."

Of experimental investigations of cholagogues made on human subjects, the preceding are all the results which we could find in the literature.

All the more recent experimental investigations of this subject made on animals have led to the result that all the supposed cholagogues,

* Loc. cit.

† Eisen und Farbstoffausscheidung in der Galle. Pflüger's *Archiv*, xv, p. 353.

‡ Loc. cit.

§ Loc. cit.

with the exception of bile itself, and possibly salicylic acid, do not increase the flow of bile, that is to say, are not cholagogues.

Bile, as is now generally taught in works on physiology, after being discharged into the duodenum, is to a certain extent reabsorbed and again used for new secretion. If this is true, bile given internally must naturally increase the flow of bile coming from a biliary fistula. This phenomenon of reabsorption of the bile is now spoken of as "circulation of bile," and to Schiff is given the credit of having established for the first time this fact. We find in perusing the older literature that in the second half of the last century the probability of reabsorption of the bile or some of its products was already discussed.* In the present century Liebig was the first to formulate precisely a theory of reabsorption of the bile from the intestinal tract. This theory seems to have been generally accepted by scientific investigators of his time. We find allusions to this theory in different places, as by E. von Gorup-Besanez,† Rheinhold Schellbach,‡ T. Neukomm,§ and especially by C. G. Lehmann.¶ Here Liebig's theory of the "circuit" of the bile "from the liver to the intestines and thence back to the liver" is very clearly put forward. It is true the theory could be only indirectly sustained by the examination of the contents of the bowel, "in various portions of the whole intestinal tract from above downwards." This examination "almost necessarily leads us to adopt the view that the greater part of the bile is again reabsorbed as it passes through the intestine and is returned to the general mass of the fluids."

The first direct experimental proof that biliary constituents if absorbed are again secreted by the liver was given by Huppert.| He determined in rabbits with fresh biliary fistulæ the biliary secretion and the amount of sodium glycocholate and taurocholate in it. After-

* Experiments upon the Human Bile and Reflections on the Biliary Secretion. By James M'Clurg. London, 1772.

† Ueber die Funktion der Galle. Liebig's *Annalen*, 1851.

‡ Untersuchungen über Galle. Liebig's *Annalen*, 1846.

§ Nachweisung der Gallensäuren und die Umwandlung derselben in der Blutbahn. Ibid. 1860.

¶ Physiological Chemistry. Translated by G. E. Day. Vol. ii, p. 110.

| Ueber das Schicksal der Gallensäuren im Icterus. *Archiv der Heilkunde*, 1864.

wards he injected bile constituents subcutaneously. He says: "It may be concluded with certainty from these observations that the liver also secretes a by no means inconsiderable fraction of the bile acids which have passed into the blood."

Only a few years later, 1868, appeared the well-known publications of M. Schiff* on this subject, and they caused much controversy. N. Socoloff,† working in Hoppe-Seyler's laboratory, denied absolutely any quantitative increase of bile constituents after injection of bile. Looking carefully over his experiments, we again find another proof that it is quite impossible to draw conclusions from experiments on animals with biliary fistulæ if these experiments are conducted for a few hours only. Socoloff collected in his first experiment during the first hour 0.247 gramme bile, and in the subsequent hour 2.212 grammes, and in another experiment with the same animal during the first hour 11.207 grammes bile, and in the subsequent hour 4.064 grammes. Such variations in the normal flow of bile are too great to allow any positive conclusion—from comparisons during such units of time—as to the action of supposed cholagogues.

It would take too long for us to give a detailed account of all the investigations in this line. We shall mention only the results obtained by Rosenkranz,‡ who experimented on dogs with complete and incomplete fistulæ. The time of observation in his experiments extended often over 24 hours. His results may be thus summarized: "Bile, if given into the stomach or into the intestine, increases the secretion of bile discharged from the fistula. There is not only an increase of the absolute quantity of bile secreted in the time units, but also an increase in the percentage of solids. The quantity of solids may increase after ingestion of bile to twice or three times its original amount. If one injects bile rich in solids, a greater amount of bile is discharged from the fistulous opening than after the ingestion of the same quantity of dilute bile."

* Gallenbildung abhängig von der Aufsaugung der Gallenstoffe. Pflüger's *Archiv*, iii, p. 598.

† Ein Beitrag zur Kenntniss der Lebersekretion. Pflüger's *Archiv*, xi, p. 166.

‡ Ueber das Schicksal und Bedeutung einiger Gallenbestandtheile. *Vrhdlg. der physikal. medic. Gesellschaft in Würzburg*, xiii, p. 218.

Objections may be made to conclusions drawn from the percentage of solids of a bile as to the supposed amount of glycocholic and taurocholic acid in it, such conclusions being misleading.

In our experiments we not only determined the amount of bile solids in each sample of bile, but also the amount of ash, to establish an absolute proof of a supposed increase of combustible substances. But as such substances need not necessarily be, and as we shall see are not always formed by characteristic bile elements, we determined for different series of experiments the quantity of glycocholic and taurocholic acid found in the bile of the corresponding periods.

As we had concluded to try bile on our patient for its supposed cholagogue action, the question arose, what bile should we give, in what form and to what extent? That bile circulating in excess in the system has a depressing action on the circulatory system is a well-known fact which has been observed abundantly on patients suffering from jaundice and also after direct experiments on animals.* Besides this, the nauseating and purging effect of bile had to be avoided if the results to be obtained were to be trustworthy.

We thought that by giving dried bile in pill form the nauseating taste and smell would be avoided.

What kind of bile should we give? There is no positive proof, nor even a strong probability, that bile of different animals—in spite of its varying amount of the characteristic bile acids—would act differently. Nevertheless we find it stated by different experimenters that animals react differently to different biles. As we had of course to use the utmost caution with our patient, we thought it wise to try first the effect of her own bile on her system before resorting to bile of other origin.

In what quantities should we give the bile? This depended entirely on the question—to what extent is bile reabsorbed from the intestine? We do not know. If given in small quantities, the amount of reabsorbed bile may be so small that the difference in the total quantities of bile excreted through the biliary fistula might be regarded as a normally occurring variation, and the supposed cholagogue action could

* Roehrig, Ueber den Einfluss der Galle auf die Herzthätigkeit. *Archiv der Heilkunde*, 1863, p. 385.

not be positively demonstrated. We reasoned that the amount of bile which our patient secreted daily was the minimum amount of bile which would physiologically reach the intestines. We say the minimum amount, as it is a well-established fact that bile gained by a fistula has always less solids than normal bile. In our case the patient was not reabsorbing bile for over two months at least, that is to say since the date of the operation. Therefore the amount of bile collected from the fistula during 24 hours could be regarded as representing the total quantity of fresh bile formed daily in our subject. Whether the same quantity of fresh bile is formed daily when reabsorption of bile is possible is of course impossible to determine. To imitate nature we had therefore to give to our patient daily at least the same quantity of bile she discharged through the biliary fistula. For obvious reasons we began to give her at first much smaller quantities, but we increased the amount gradually, until finally she was given *per os* daily nearly the equivalent of bile which was discharged *per fistulam*.

We evaporated her own fresh bile on the water-bath till the residue was perfectly dry. Out of this residue pills were made with the addition of the necessary amount of glycerine, so that each pill corresponded exactly to 25 cc. of liquid bile. The largest number of pills taken was twenty per day, which corresponded to 500 cc. of her fresh bile, whereas she discharged on the average, during 24 hours, 514.3 cc. bile. As to the results of our experiments with human bile, we shall record these with all the other results in the subsequent table, and to make them more plainly visible also represent them in a curve (Plate IX).

Beside the effect of human bile, we studied the action of salol, sublimate, ox bile, calomel, and a mixture of pure salts of glycocholic and taurocholic acid prepared from ox bile.

Calomel is still employed largely in some countries for its supposed cholagogue effect, as is also corrosive sublimate. Salol was given as a supposed cholagogue instead of salicylic acid, as we wished to learn how it acted, so that we might, if necessary, use this substance to coat pills of ox bile in case that the taste of the latter should be disagreeable to the patient, or that we should find it necessary to prevent

absorption of drugs already in the stomach. Pills coated with salol, if properly made, pass unaltered out of the stomach* and reach the intestine, where the alkaline succus entericus decomposes the salol into salicylic acid and phenol, and thus allows the contents of the pills free action.

To avoid repetition we wish to state that the patient, during the whole time of observation, lived on the ordinary hospital diet, a table of which has been given on page 52. The time of the meals was always the same: breakfast at 7 A. M.; dinner, 1 P. M.; and supper, 6 P. M. The patient during the whole time of the experiments felt perfectly well. Any objective signs or subjective symptoms—at one time a kind of neuralgic pain in the teeth, and at another time soreness of the gums—will be found noted in the records of the day when they occurred.

Menstruation of the patient was always regular† before and after the operation. At the beginning of our investigation the patient had a dislike for fatty foods and butter. In fact she left a portion of the daily allowance of butter uneaten. If the patient walked about the ward the movements of the bowels were fairly regular; when she stayed in bed she became constipated. The fæces had a very bad odor and were clay-colored. No bile constituents could be detected in them nor in the urine.

The mass of fæces passed was enormous. Judging from their size one would suppose the patient would have had difficulty, or at least much discomfort, in passing them. But she never complained of this. The reason why she had no inconvenience in passing such stools may be explained by the fact that the fæces contained a large amount of fat, which, if warmed to body temperature, becomes soft. Patients with icterus‡ often say that they have a soft stool, when the fæcal matter, after standing, has a perfectly natural shape.

* G. Oeder, Salolüberzug für Dünndarmpillen. *Berlin. klin. Wochenschrift*, 1894, No. 15.

† M. Robson's patient (l. c.) did not menstruate so long as the fistula was patent. As soon as the bile was again turned into the intestine, the menstrual function became regular and normal.

‡ F. Müller, Untersuchungen über Icterus. *Zeitschr. f. klin. Medicin*, xii, p. 45 *et seq.*

From January 12 until April 18 the bile discharged from the fistulous opening was constantly collected. Every six hours, with the exception of the third day of our observation, the receiving glass—a graduated cylinder—was changed. The bile was kept well stoppered—from one day to another—in a cool place, and each day at 2 P. M. brought to the laboratory—twenty minutes' walk—where the quantities were measured and the different samples directly analyzed. A check table of the quantities was always kept in the hospital, so that in case one bottle should break during transport, at least the quantity for the corresponding six hours could be stated. The time when the graduated cylinders were changed was always 2 P. M., 8 P. M., 2 A. M. and 8 A. M., the day counting from 2 P. M. on one day to 2 P. M. the next.

The first day in the following table (Table II) was from 2 P. M., January 12, until 2 P. M., January 13; and the last day of observation, the 97th day in the curve, was from the 17th to the 18th of April.

The specific gravity of each sample of bile was taken at the temperature of the laboratory with the same hydrometer. To determine the solids and the ash, 25 cc. of bile were evaporated in a platinum dish on the water-bath, and afterwards dried at 105–110° C. to constant weight. The platinum dish containing the solids was now heated in a muffle, kept as nearly as possible at the same temperature. As soon as the bile began to char, a burning match was held over the dish so that the bile took fire. It seemed to us that in this way we got more quickly an ash free from carbon. As soon as the ash ceased to diminish in weight, when kept half an hour at a dull red heat, the ignition was concluded. The quantitative determination of ash in bile is never entirely trustworthy.* It was to reduce the error that we determined the ash in each sample of bile collected every six hours, instead of making only one analysis on the mixed bile for each day.

In the following table will be found all the analyses of solids and ash of the different samples of bile collected during our observation.

*Hoppe-Seyler, Handbuch der physiol. und pathol. chemischen Analyse, p. 448, 1893.

TABLE II.

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
	2 P. M.–8 P. M.	143	1.009	0.4020	0.2005						
	8 P. M.–2 A. M.	110	1.009	0.4292	0.1558						
	2 A. M.–8 A. M.	84	1.010	0.5025	0.2260						
1	8 A. M.–2 P. M.	180	1.009	0.3933	0.2146	517	8.7079	4.1367	1.68	.800	No medication.
	2 P. M.–8 P. M.	148	1.008	0.3658	0.2111						
	8 P. M.–2 A. M.	94	1.008	0.4205	0.2277						
	2 A. M.–8 A. M.	92	1.008	0.4690	0.2180						
2	8 A. M.–2 P. M.	167	1.008	0.3865	0.1988	501	8.0542	4.2359	1.61	.85	No medication.
	.....	....	....	....	....						
	.....	....	....	....	....						
	.....	....	....	....	....						
3	2 P. M.–2 P. M.	525	1.009	0.4261	0.2243	525	8.9481	4.7103	1.70	.90	No medication.
	2 P. M.–8 P. M.	141	1.009	0.4028	0.1989						
	8 P. M.–2 A. M.	108	1.010	0.4295	0.2305						
	2 A. M.–8 A. M.	134	1.009	0.4450	0.2148						
4	8 A. M.–2 P. M.	162	1.009½	0.4058	0.2143	545	9.1418	4.6573	1.68	.85	Medication, human bile. 3 pills.
	2 P. M.–8 P. M.	174	1.008	0.4203	0.2287						
	8 P. M.–2 A. M.	131	1.008	0.4771	0.2263						
	2 A. M.–8 A. M.	106	1.009	0.5379	0.2271						
5	8 A. M.–2 P. M.	175	1.009	0.4098	0.2315	586	10.5744	5.3609	1.80	.91	" " 6 pills.
	2 P. M.–8 P. M.	155	1.008	0.4233	0.2343						
	8 P. M.–8 A. M.	172	1.008	0.5333	0.2198						
	.....	....	....	....	....						
6	8 A. M.–2 P. M.	202	1.008	0.4080	0.2259	529	9.6521	4.7900	1.82	.91	" " 6 pills.
	2 P. M.–8 P. M.	150	1.009	0.4082	9.2294						
	8 P. M.–2 A. M.	107	1.009	0.4627	0.2115						
	2 A. M.–8 A. M.	84	1.010	0.5463	0.2175						
7	8 A. M.–2 P. M.	188	1.010	0.4053	0.2079	529	9.3128	4.5758	1.76	.86	" " 9 pills.
	2 P. M.–8 P. M.	171	1.008½	0.4182	0.2283						
	8 P. M.–2 A. M.	103	1.010	0.4835	0.2237						
	2 A. M.–8 A. M.	123	1.008	0.4978	0.2225						
8	8 A. M.–2 P. M.	203	1.008½	0.4262	0.2320	600	10.7622	5.4616	1.79	.91	" " 12 pills.
	2 P. M.–8 P. M.	173	1.008½	0.4646	0.2283						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids	% ash.	Remarks.
	8 P. M.–2 A. M.	96	1.010	0.5384	0.2300						Medication, human bile.
	2 A. M.–8 A. M.	149	1.010½	0.5295	0.2234						
9	8 A. M.–2 P. M.	156	1.009	0.4105	0.2307	574	10.9997	5.2339	1.92	.91	" " 15 pills.
	2 P. M.–8 P. M.	174	1.008	0.4402	0.2330						
	8 P. M.–2 A. M.	128	1.008½	0.4679	0.2284						
	2 A. M.–8 A. M.	133	1.009	0.4975	0.2249						
10	8 A. M.–2 P. M.	236	1.010	0.4697	0.2325	671	12.3399	6.1822	1.84	.92	" " 18 pills.
	2 P. M.–8 P. M.	199	1.009	0.5302	0.2331						
	8 P. M.–2 A. M.	107	1.009	0.5172	0.2220						
	2 A. M.–8 A. M.	112	1.010	0.6312	0.2227						
11	8 A. M.–2 P. M.	189	1.008½	0.4091	0.2355	607	12.3543	5.5834	2.04	.92	" " 20 pills.
	2 P. M.–8 P. M.	221	1.009	0.4667	0.2219						
	8 P. M.–2 A. M.	139	1.009	0.5130	0.2292						
	2 A. M.–8 A. M.	132	1.009½	0.5329	0.2268						
12	8 A. M.–2 P. M.	193	1.010	0.4607	0.2290	685	12.3481	6.2011	1.80	.91	" " 20 pills.
	2 P. M.–8 P. M.	204	1.008½	0.5129	0.2364						
	8 P. M.–2 A. M.	102	1.009	0.5391	0.2265						
	2 A. M.–8 A. M.	114	1.010	0.5907	0.2195						
13	8 A. M.–2 P. M.	202	1.008	0.3875	0.2304	622	12.2092	5.7156	1.96	.92	" " 20 pills.
	2 P. M.–8 P. M.	172	1.008½	0.4917	0.2259						
	8 P. M.–2 A. M.	150	1.009½	0.5177	0.2326						
	2 A. M.–8 A. M.	98	1.009½	0.5472	0.2237						
14	8 A. M.–2 P. M.	193	1.010	0.4053	0.2288	613	11.7629	5.5939	1.92	.91	" " 20 pills.
	2 P. M.–8 P. M.	190	1.008	0.4720	0.2252						
	8 P. M.–2 A. M.	130	1.009½	0.5085	0.2274						
	2 A. M.–8 A. M.	139	1.008½	0.4852	0.2242						
15	8 A. M.–2 P. M.	197	1.008	0.4101	0.2111	656	12.1606	5.8038	1.85	.88	" " 19 pills.
	2 P. M.–8 P. M.	172	1.008	0.4715	0.2285						
	8 P. M.–2 A. M.	113	1.008	0.4487	0.2214						
	2 A. M.–8 A. M.	129	1.008½	0.4973	0.2230						
16	8 A. M.–2 P. M.	144	1.009	0.4350	0.2256	558	10.3436	5.0228	1.85	.90	No medication.
	2 P. M.–8 P. M.	128	1.008½	0.4332	0.2222						
	8 P. M.–2 A. M.	137	1.009	0.4676	0.2276						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
	2 A. M.–8 A. M.	90	1.008½	0.4601	0.2123						
17	8 A. M.–2 P. M.	149	1.008½	0.3929	0.2177	504	8.7782	4.4464	1.74	.88	No medication.
	2 P. M.–8 P. M.	84	1.008½	0.4444	0.2299						
	8 P. M.–2 A. M.	96	1.008½	0.4786	0.2172						
	2 A. M.–8 A. M.	136	1.008½	0.4045	0.2222						
18	8 A. M.–2 P. M.	170	1.008	0.3938	0.2223	486	8.2091	4.3267	1.69	.89	" "
	2 P. M.–8 P. M.	129	1.008	0.4069	0.2128						
	8 P. M.–2 A. M.	70	1.008½	0.5072	0.1944						
	2 A. M.–8 A. M.	105	1.009	0.5202	0.2212						
19	8 A. M.–2 P. M.	133	1.008½	0.4045	0.2208	437	7.8564	3.7459	1.80	.85	" "
	2 P. M.–8 P. M.	150	1.008	0.3805	0.2099						
	8 P. M.–2 A. M.	57	1.008	0.4489	0.2170						
	2 A. M.–8 A. M.	84	1.009	0.4875	0.2030						
20	8 A. M.–2 P. M.	167	1.007½	0.3529	0.1954	458	7.3017	3.7413	1.59	.82	Medication, salol. 4 × 0.3.
	2 P. M.–8 P. M.	122	1.008	0.3957	0.2205						
	8 P. M.–2 A. M.	54	1.009	0.4183	....						
	2 A. M.–8 A. M.	122	1.008½	0.4434	0.2092						
21	8 A. M.–2 P. M.	161	1.008	0.3956	0.2258	459	7.5474	....	1.64	..	" " 4 × 0.6.
	2 P. M.–8 P. M.	164	1.007½	0.3968	0.2064						
	8 P. M.–2 A. M.	88	1.008½	0.4638	0.2035						
	2 A. M.–8 A. M.	92	1.009	0.4703	0.2173						
22	8 A. M.–2 P. M.	190	1.008	0.3781	0.2030	534	8.8397	4.4100	1.66	.83	" " "
	2 P. M.–8 P. M.	126	1.008	0.4427	0.1996						
	8 P. M.–2 A. M.	111	1.008½	0.4235	0.2205						
	2 A. M.–8 A. M.	129	1.008½	0.3715	0.2031						
23	8 A. M.–2 P. M.	164	1.008	0.4047	0.2288	530	8.6832	4.5337	1.64	.86	" " "
	2 P. M.–8 P. M.	132	1.008	0.4160	0.1963						
	8 P. M.–2 A. M.	126	1.008	0.4796	0.2007						
	2 A. M.–8 A. M.	101	1.009	0.4823	0.1812						
24	8 A. M.–2 P. M.	182	1.008	0.4049	0.1939	541	9.5095	4.1914	1.76	.77	" " 4 × 1.2.
	2 P. M.–8 P. M.	160	1.008½	0.4042	0.2202						
	8 P. M.–2 A. M.	112	1.008½	0.4801	0.2087						
	2 A. M.–8 A. M.	98	1.008	0.4846	0.2118						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
25	8 A. M.–2 P. M.	168	1.008	0.3937	0.2152	538	9.2828	4.6204	1.73	.86	Medication, salol. 4×1.2.
	2 P. M.–8 P. M.	180	1.008	0.3937	0.2227						
	8 P. M.–2 A. M.	110	1.009	0.4942	0.1817						
	2 A. M.–8 A. M.	86	1.009	0.4967	0.2011						
26	8 A. M.–2 P. M.	167	1.009	0.4192	0.1769	543	9.5178	4.2861	1.75	.79	" " 3×1.2.
	2 P. M.–8 P. M.	150	1.009	0.4324	0.2025						
	8 P. M.–2 A. M.	154	1.010	0.4703	0.2110						
	2 A. M.–8 A. M.	92	1.010	0.4978	0.1808						
27	8 A. M.–2 P. M.	152	1.009	0.4143	0.1992	548	9.8422	4.3911	1.80	.80	No medication.
	2 P. M.–8 P. M.	74	1.008	0.4153	0.2124						
	8 P. M.–2 A. M.	135	1.009	0.4699	0.1992						
	2 A. M.–8 A. M.	116	1.009½	0.5023	0.2146						
28	8 A. M.–2 P. M.	163	1.008	0.4157	0.2149	488	8.8075	4.1003	1.80	.84	" "
	2 P. M.–8 P. M.	152	1.008	0.4183	0.2166						
	8 P. M.–2 A. M.	96	1.009	0.4826	0.1820						
	2 A. M.–8 A. M.	75	1.008½	0.4956	0.2075						
29	8 A. M.–2 P. M.	163	1.008	0.3907	0.1683	486	8.4304	3.7355	1.73	.77	Medication, corrosive sublimate. 4×0.0015.
	2 P. M.–8 P. M.	141	1.008	0.3957	0.2279						
	8 P. M.–2 A. M.	92	1.008½	0.4579	0.1723						
	2 A. M.–8 A. M.	100	1.009	0.4980	0.1899						
30	8 A. M.–2 P. M.	152	1.009	0.3928	0.2271	485	8.2969	4.0596	1.71	.84	" " "
	2 P. M.–8 P. M.	169	1.007½	0.4132	0.2298						
	8 P. M.–2 A. M.	81	1.009	0.4672	0.2178						
	2 A. M.–8 A. M.	87	1.010	0.4887	0.1832						
31	8 A. M.–2 P. M.	180	1.008½	0.3903	0.2270	517	8.8176	4.5309	1.71	.88	" " "
	2 P. M.–8 P. M.	166	1.008	0.3927	0.2044						
	8 P. M.–2 A. M.	105	1.008½	0.4556	0.2266						
	2 A. M.–8 A. M.	82	1.009½	0.5065	0.2261						
32	8 A. M.–2 P. M.	162	1.008	0.4005	0.2317	515	8.7775	4.5519	1.71	.88	" " "
	2 P. M.–8 P. M.	163	1.008	0.3873	0.2224						
	8 P. M.–2 A. M.	114	1.009½	0.4750	0.2243						
	2 A. M.–8 A. M.	69	1.009	0.4790	0.1997						
33	8 A. M.–2 P. M.	152	1.009	0.4243	0.2159	498	8.4928	4.3366	1.71	.87	" " "

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
	2 P. M.–8 P. M.	147	1.008	0.4367	0.2286						
	8 P. M.–2 A. M.	106	1.008½	0.4134	0.2225						
	2 A. M.–8 A. M.	110	1.009	0.4606	0.2230						
34	8 A. M.–2 P. M.	126	1.009	0.4016	0.2150	489	8.3750	4.3568	1.71	.90	Medication, corrosive sublimate. 4 × 0.003
	2 P. M.–8 P. M.	136	1.008½	0.4013	0.2263						
	8 P. M.–2 A. M.	124	1.009	0.4164	0.2259						
	2 A. M.–8 A. M.	98	1.009½	0.4591	0.2213						
35	8 A. M.–2 P. M.	155	1.009	0.4003	0.2204	513	8.5239	4.5806	1.66	.89	" " "
	2 P. M.–8 P. M.	146	1.009½	0.3865	0.2264						
	8 P. M.–2 A. M.	93	1.008½	0.4468	0.2258						
	2 A. M.–8 A. M.	109	1.010	0.4961	0.2210						
36	8 A. M.–2 P. M.	189	1.010	0.4023	0.2206	537	9.1244	3.7947	1.70	.71	No medication.
	2 P. M.–8 P. M.	147	1.008½	0.3910	0.2302						
	8 P. M.–2 A. M.	109	1.009	0.4813	0.2252						
	2 A. M.–8 A. M.	....	1.009	0.5536	0.2219						
37	8 A. M.–2 P. M.	160	1.008½	0.3961	0.2266	..	....	....	..	..	" "
	2 P. M.–8 P. M.	143	1.008	0.4071	0.2218						
	8 P. M.–2 A. M.	109	1.008½	0.4746	0.2235						
	2 A. M.–8 A. M.	110	1.009½	0.4778	0.2282						
38	8 A. M.–2 P. M.	177	1.008	0.4070	0.2305	539	9.3837	4.8848	1.74	.91	" "
	2 P. M.–8 P. M.	135	1.009	0.4251	0.2289						
	8 P. M.–2 A. M.	114	1.009	0.4620	0.2245						
	2 A. M.–8 A. M.	69	1.010½	0.5378	0.2253						
39	8 A. M.–2 P. M.	165	1.008	0.4041	0.2382	483	8.5529	4.4544	1.77	.92	" "
	2 P. M.–8 P. M.	160	1.008	0.3906	0.2255						
	8 P. M.–2 A. M.	114	1.008	0.4392	0.2233						
	2 A. M.–8 A. M.	91	1.008½	0.4876	0.2283						
40	8 A. M.–2 P. M.	156	1.008	0.4055	0.2292	521	8.8139	4.7220	1.69	.91	" "
	2 P. M.–8 P. M.	146	1.009	0.4024	0.2330						
	8 P. M.–2 A. M.	175	1.009½	0.5130	0.2352						
	2 A. M.–8 A. M.	144	1.010½	0.6199	0.2335						
41	8 A. M.–2 P. M.	229	1.010	0.5983	0.2315	694	15.0050	6.4786	2.16	.93	Medication, ox bile. 12 pills.
	2 P. M.–8 P. M.	192	1.009	0.5788	0.2363						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
	8 P. M.–2 A. M.	145	1.009½	0.6181	0.2141						
	2 A. M.–8 A. M.	138	1.011	0.8702	0.2268						
42	8 A. M.–2 P. M.	209	1.008½	0.5348	0.2223	684	17.3061	6.1625	2.53	.90	Medication, ox bile. 12 pills.
	2 P. M.–8 P. M.	186	1.009½	0.6356	0.2308						
	8 P. M.–2 A. M.	145	1.010	0.6355	0.2244						
	2 A. M.–8 A. M.	145	1.010	0.6632	0.2242						
43	8 A. M.–2 P. M.	238	1.011	0.6738	0.2297	714	18.6824	6.5066	2.62	.91	" " 16 pills.
	2 P. M.–8 P. M.	190	1.010	0.5851	0.2341						
	8 P. M.–2 A. M.	116	1.011	0.5871	0.2210						
	2 A. M.–8 A. M.	184	1.009½	0.5944	0.2327						
44	8 A. M.–2 P. M.	210	1.009½	0.5743	0.2309	700	16.3630	6.4590	2.34	.92	" " "
	2 P. M.–8 P. M.	218	1.009½	0.6189	0.2344						
	8 P. M.–2 A. M.	150	1.011	0.6069	0.2333						
	2 A. M.–8 A. M.	163	1.010½	0.6329	0.2215						
45	8 A. M.–2 P. M.	229	1.010½	0.6667	0.2307	760	19.2764	7.0017	2.54	.92	" " "
	2 P. M.–8 P. M.	229	1.010	0.6323	0.2201						
	8 P. M.–2 A. M.	150	1.009	0.6080	0.2179						
	2 A. M.–8 A. M.	146	1.009½	0.6923	0.2243						
46	8 A. M.–2 P. M.	194	1.009½	0.5659	0.2330	719	17.8704	6.4393	2.49	.89	" " "
	2 P. M.–8 P. M.	214	1.010	0.5967	0.2303						
	8 P. M.–2 A. M.	160	1.010	0.6105	0.2227						
	2 A. M.–8 A. M.	176	1.010	0.6009	0.2237						
47	8 A. M.–2 P. M.	220	1.010	0.6493	0.2084	770	18.9655	6.8033	2.46	.88	" " 20 pills.
	2 P. M.–8 P. M.	214	1.009½	0.5900	0.2185						
	8 P. M.–2 A. M.	157	1.010	0.6024	0.2255						
	2 A. M.–8 A. M.	121	1.009	0.5813	0.2130						
48	8 A. M.–2 P. M.	154	1.008½	0.4853	0.2131	646	14.6330	5.6117	2.27	.87	No medication.
	2 P. M.–8 P. M.	140	1.008½	0.4750	0.1709						
	8 P. M.–2 A. M.	108	1.009	0.5073	0.1988						
	2 A. M.–8 A. M.	70	1.009½	0.5089	0.2034						
49	8 A. M.–2 P. M.	84	1.008	0.4092	0.2168	402	7.6496	3.1147	1.90	.77	" "
	2 P. M.–8 P. M.	131	1.007½	0.4131	0.2293						
	8 P. M.–2 A. M.	101	1.008	0.4443	0.2067						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.	
	2 A. M.–8 A. M.	114	1.008½	0.4390	0.2102							
50	8 A. M.–2 P. M.	150	1.008	0.3563	0.2285	496	8.0956	4.3677	1.63	.88	No medication.	
	2 P. M.–8 P. M.	125	1.008½	0.3873	0.2253							
	8 P. M.–2 A. M.	82	1.010	0.4730	0.2189							
	2 A. M.–8 A. M.	122	1.010	0.4654	0.2174							
51	8 A. M.–2 P. M.	160	1.009½	0.3647	0.2194	489	8.0852	4.3038	1.66	.88	" "	
	2 P. M.–8 P. M.	126	1.008	0.3832	0.2285							
	8 P. M.–2 A. M.	....	1.008	0.4130	0.2190							
	2 A. M.–8 A. M.	96	1.009	0.4725	0.2203							
52	8 A. M.–2 P. M.	162	1.008	0.3898	0.2216	..	....	....	..	..	" "	
	2 P. M.–8 P. M.	127	1.008	0.3952	0.2244							
	8 P. M.–2 A. M.	82	1.008½	0.4301	0.2102							
	2 A. M.–8 A. M.	138	1.009	0.4230	0.2079							
53	8 A. M.–2 P. M.	155	1.008½	0.3917	0.2163	502	8.1823	4.3140	1.63	.86	Medication, calomel.	3 × 0.03.
	2 P. M.–8 P. M.	124	1.008	0.4027	0.2159							
	8 P. M.–2 A. M.	108	1.008	0.4500	0.2108							
	2 A. M.–8 A. M.	93	1.009	0.5077	0.2087							
54	8 A. M.–2 P. M.	152	1.008½	0.3427	0.2007	477	7.9179	3.9822	1.66	.83	" "	4 × 0.03.
	2 P. M.–8 P. M.	124	1.009	0.3985	0.2199							
	8 P. M.–2 A. M.	96	1.010	0.4322	0.2172							
	2 A. M.–8 A. M.	103	1.009	0.4567	0.2254							
55	8 A. M.–2 P. M.	152	1.008	0.4079	0.1989	475	8.0012	4.0613	1.68	.86	" "	5 × 0.03.
	2 P. M.–8 P. M.	149	1.008	0.4090	0.2150							
	8 P. M.–2 A. M.	96	1.008½	0.4225	0.1948							
	2 A. M.–8 A. M.	95	1.009½	0.5119	0.2099							
56	8 A. M.–2 P. M.	151	1.008	0.3922	0.2256	491	8.3751	4.1932	1.71	.85	" "	6 × 0.03.
	2 P. M.–8 P. M.	128	1.008½	0.4012	0.2032							
	8 P. M.–2 A. M.	85	1.009	0.4405	0.2186							
	2 A. M.–8 A. M.	104	1.009½	0.4884	0.2168							
57	8 A. M.–2 P. M.	162	1.008½	0.3801	0.2228	479	8.0449	4.1316	1.68	.86	" "	7 × 0.03.
	2 P. M.–8 P. M.	161	1.008½	0.3949	0.2202							
	8 P. M.–2 A. M.	87	1.009½	0.4577	0.2245							
	2 A. M.–8 A. M.	129	1.009½	0.4417	0.2228							

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
58	8 A. M.–2 P. M.	134	1.008½	0.3990	0.2231	511	8.5569	4.5456	1.68	.89	Medication, calomel. 4 × 0.03.
	2 P. M.–8 P. M.	139	1.008	0.3915	0.2211						
	8 P. M.–2 A. M.	78	1.009	0.4521	0.2158						
	2 A. M.–8 A. M.	77	1.010	0.5357	0.2218						
59	8 A. M.–2 P. M.	150	1.009	0.3952	0.2267	444	7.6105	3.5397	1.71	.80	No medication.
	2 P. M.–8 P. M.	148	1.008½	0.3806	0.2245						
	8 P. M.–2 A. M.	92	1.009	0.4455	0.2142						
	2 A. M.–8 A. M.	98	1.009¼	0.4941	0.2040						
60	8 A. M.–2 P. M.	148	1.008	0.4022	0.2286	486	8.2129	4.2747	1.69	.88	" "
	2 P. M.–8 P. M.	151	1.009	0.3970	0.2215						
	8 P. M.–2 A. M.	78	1.010	0.4326	0.2204						
	2 A. M.–8 A. M.	111	1.010	0.4764	0.2216						
61	8 A. M.–2 P. M.	152	1.009¼	0.4208	0.2227	492	8.4217	4.3686	1.71	.88	" "
	2 P. M.–8 P. M.	154	1.009¼	0.3990	0.2149						
	8 P. M.–2 A. M.	82	1.010	0.4741	0.2150						
	2 A. M.–8 A. M.	120	1.010	0.4833	0.2033						
62	8 A. M.–2 P. M.	146	1.009¼	0.3975	0.2251	502	8.6552	4.3180	1.70	.86	" "
	2 P. M.–8 P. M.	140	1.009¼	0.3968	0.2284						
	8 P. M.–2 A. M.	77	1.009	0.4379	0.2191						
	2 A. M.–8 A. M.	102	1.009½	0.4759	0.2103						
63	8 A. M.–2 P. M.	147	1.009	0.3957	0.2074	466	7.8426	4.0252	1.68	.86	" "
	2 P. M.–8 P. M.	136	1.008	0.4089	0.2279						
	8 P. M.–2 A. M.	102	1.009	0.4388	0.2280						
	2 A. M.–8 A. M.	115	1.009	0.4593	0.2265						
64	8 A. M.–2 P. M.	158	1.008	0.4130	0.2270	511	8.7375	4.6493	1.71	.91	" "
	2 P. M.–8 P. M.	142	1.009	0.4121	0.2157						
	8 P. M.–2 A. M.	78	1.009½	0.4675	0.2265						
	2 A. M.–8 A. M.	106	1.009¼	0.4741	0.2130						
65	8 A. M.–2 P. M.	154	1.009	0.3984	0.2027	480	8.2615	4.0885	1.72	.85	" "
	2 P. M.–8 P. M.	146	1.008¼	0.3896	0.2102						
	8 P. M.–2 A. M.	72	1.009½	0.4648	0.2157						
	2 A. M.–8 A. M.	108	1.010	0.4905	0.2157						
66	8 A. M.–2 P. M.	138	1.009	0.4026	0.2047	464	7.9624	3.9131	1.72	.84	" "

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
	2 P. M.–8 P. M.	107	1.008	0.3964	0.2130						
	8 P. M.–2 A. M.	100	1.009	0.4213	0.2189						
	2 A. M.–8 A. M.	105	1.010¼	0.4740	0.2162						
67	8 A. M.–2 P. M.	154	1.009	0.3932	0.2077	466	7.7904	3.9760	1.67	.85	No medication.
	2 P. M.–8 P. M.	139	1.008½	0.4065	0.1840						
	8 P. M.–2 A. M.	108	1.009¼	0.4487	0.1683						
	2 A. M.–8 A. M.	122	1.010	0.4574	0.1957						
68	8 A. M.–2 P. M.	133	1.010	0.3976	0.2261	502	8.5499	3.9074	1.70	.78	" "
	2 P. M.–8 P. M.	167	1.008	0.3881	0.1778						
	8 P. M.–2 A. M.	....	....	....	....						
	2 A. M.–8 A. M.	87	1.009¼	0.4668	0.1851						
69	8 A. M.–2 P. M.	140	1.009½	0.4110	0.1944	..	....	....	..	..	" "
	2 P. M.–8 P. M.	116	1.008	0.3987	0.1782						
	8 P. M.–2 A. M.	89	1.009¼	0.4319	0.2130						
	2 A. M.–8 A. M.	119	1.008½	0.4450	0.1854						
70	8 A. M.–2 P. M.	166	1.008½	0.3927	0.1774	490	8.1169	3.6399	1.66	.74	" "
	2 P. M.–8 P. M.	151	1.008	0.3824	0.2114						
	8 P. M.–2 A. M.	85	1.009	0.4330	0.2083						
	2 A. M.–8 A. M.	117	1.009½	0.4639	0.1998						
71	8 A. M.–2 P. M.	169	1.008½	0.3703	0.2123	522	9.4521	3.6487	1.81	.70	" "
	2 P. M.–8 P. M.	152	1.008	0.3845	0.2216						
	8 P. M.–2 A. M.	70	1.009¼	0.4400	0.2087						
	2 A. M.–8 A. M.	86	1.010¼	0.4965	0.2145						
72	8 A. M.–2 P. M.	161	1.009¼	0.3785	0.2248	469	7.7231	4.1235	1.64	.88	" "
	2 P. M.–8 P. M.	146	1.008½	0.3795	0.2194						
	8 P. M.–2 A. M.	106	1.009	0.4099	0.2079						
	2 A. M.–8 A. M.	85	1.010¼	0.4664	0.2121						
73	8 A. M.–2 P. M.	171	1.010	0.3796	0.2058	508	8.1412	4.2906	1.60	.84	" "
	2 P. M.–8 P. M.	182	....	....	....						
	8 P. M.–2 A. M.	88	1.008	0.4264	0.2132						
	2 A. M.–8 A. M.	110	1.008½	0.4286	0.2129						
74	8 A. M.–2 P. M.	147	1.008¾	0.3860	0.2144	527	....	...	..	..	" "
	2 P. M.–8 P. M.	187	1.008	0.3804	0.2048						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
	8 P. M.–2 A. M.	95	1.009½	0.4311	0.1923						
	2 A. M.–8 A. M.	109	1.010	0.4519	0.2087						
75	8 A. M.–2 P. M.	161	1.008¼	0.3743	0.1982	552	8.8562	4.4493	1.60	.80	No medication.
	2 P. M.–8 P. M.	186	1.008	0.3847	0.1379						
	8 P. M.–2 A. M.	84	1.009	0.4525	0.1784						
	2 A. M.–8 A. M.	108	1.010	0.4823	0.2097						
76	8 A. M.–2 P. M.	155	1.008¼	0.3860	0.1723	533	8.8618	3.5983	1.66	.68	" "
	2 P. M.–8 P. M.	152	1.012	0.6551	0.1857						
	8 P. M.–2 A. M.	211	1.012¼	0.7861	0.1894						
	2 A. M.–8 A. M.	148	1.012¼	0.8310	0.1435						
77	8 A. M.–2 P. M.	190	1.010	0.5742	0.1961	701	19.8981	5.0681	2.84	.72	Medication, bile salts. 2 × 0.5.
	2 P. M.–8 P. M.	182	1.009	0.5454	0.1972						
	8 P. M.–2 A. M.	136	1.010	0.6192	....						
	2 A. M.–8 A. M.	142	1.010	0.6078	0.1937						
78	8 A. M.–2 P. M.	194	1.010	0.4928	0.2019	654	14.6211	....	2.24	..	" " 4 × 0.25
	2 P. M.–8 P. M.	162	1.008¼	0.4273	0.1680						
	8 P. M.–2 A. M.	90	1.009¾	0.4897	0.2175						
	2 A. M.–8 A. M.	154	1.009	0.4964	0.2094						
79	8 A. M.–2 P. M.	181	1.008	0.4358	....	587	10.7428	....	1.83	..	" " "
	2 P. M.–8 P. M.	165	1.008¾	0.3931	0.1967						
	8 P. M.–2 A. M.	101	1.009¼	0.4425	0.1946						
	2 A. M.–8 A. M.	120	1.010	0.4370	0.1851						
80	8 A. M.–2 P. M.	192	1.010¼	0.3895	0.2141	578	9.4763	4.6195	1.64	.80	" " "
	2 P. M.–8 P. M.	156	1.008¼	0.4070	0.2100						
	8 P. M.–2 A. M.	93	1.009½	0.4355	0.1960						
	2 A. M.–8 A. M.	125	1.009	0.4282	0.1841						
81	8 A. M.–2 P. M.	176	1.009¼	0.3786	0.2253	550	8.9696	4.5435	1.63	.83	" " "
	2 P. M.–8 P. M.	168	1.008¼	0.3930	0.1942						
	8 P. M.–2 A. M.	111	1.010¼	0.4402	0.2183						
	2 A. M.–8 A. M.	120	1.010½	0.4839	0.2090						
82	8 A. M.–2 P. M.	179	1.009	0.3780	0.1559	578	9.6241	4.4203	1.67	.78	" " "
	2 P. M.–8 P. M.	198	1.008¼	0.3979	0.2069						
	8 P. M.–2 A. M.	122	1.009¼	0.4821	0.2097						

TABLE II.—*Continued.*

	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.		
	2 A. M.–8 A. M.	106	1.010¼	0.4760	0.2233								
83	8 A. M.–2 P. M.	188	1.009¾	0.3944	0.2287	614	10.4852	5.3317	1.71	.87	Medication, bile salts.		4 × 0.25.
	2 P. M.–8 P. M.	164	1.008¼	0.4257	0.1860								
	8 P. M.–2 A. M.	114	1.009¼	0.4423	0.1860								
	2 A. M.–8 A. M.	103	1.010½	0.5045	0.1959								
84	8 A. M.–2 P. M.	189	1.010	0.3775	0.2038	570	9.7482	4.4179	1.70	.78	"	"	"
	2 P. M.–8 P. M.	179	1.008	0.4209	0.2157								
	8 P. M.–2 A. M.	94	1.009	0.4405	0.1875								
	2 A. M.–8 A. M.	120	1.009	0.4702	0.1997								
85	8 A. M.–2 P. M.	194	1.009	0.4055	0.2120	587	10.0789	4.8584	1.72	.83	"	"	"
	2 P. M.–8 P. M.	188	1.008¼	0.4288	0.1601								
	8 P. M.–2 A. M.	65	1.013¼	0.7809	0.2630								
	2 A. M.–8 A. M.	153	1.009	0.4874	0.2211								
86	8 A. M.–2 P. M.	131	1.009½	0.4321	0.2210	537	10.5006	4.3975	1.95	.82	"	"	"
	2 P. M.–8 P. M.	183	1.009½	0.4294	0.2069								
	8 P. M.–2 A. M.	110	1.009¼	0.4026	0.1835								
	2 A. M.–8 A. M.	129	1.009¾	0.4556	0.2096								
87	8 A. M.–2 P. M.	169	1.009¾	0.4307	0.1774	591	10.1798	4.6049	1.72	.78	"	"	5 × 0.25.
	2 P. M.–8 P. M.	159	1.008¼	0.4429	0.2122								
	8 P. M.–2 A. M.	98	1.009¼	0.4830	0.2085								
	2 A. M.–8 A. M.	127	1.009¾	0.5138	0.1809								
88	8 A. M.–2 P. M.	181	1.010	0.4489	0.1897	565	10.5725	4.4625	1.87	.79	"	"	6 × 0.25.
	2 P. M.–8 P. M.	196	1.009	0.4491	0.1622								
	8 P. M.–2 A. M.	97	1.010	0.5142	0.1786								
	2 A. M.–8 A. M.	146	1.010¼	0.4911	0.1814								
89	8 A. M.–2 P. M.	187	1.010	0.4431	0.2155	626	11.6954	4.6370	1.87	.74	"	"	7 × 0.25.
	2 P. M.–8 P. M.	191	1.008¼	0.4855	0.1932								
	8 P. M.–2 A. M.	88	1.009¼	0.5270	0.2216								
	2 A. M.–8 A. M.	112	1.010¼	0.5220	0.2271								
90	8 A. M.–2 P. M.	186	1.009½	0.4723	0.1935	577	11.4181	4.7124	1.98	.82	"	"	8 × 0.25.
	2 P. M.–8 P. M.	190	1.010	0.5196	0.2290								
	8 P. M.–2 A. M.	111	1.010	0.5619	0.2325								
	2 A. M.–8 A. M.	116	1.010	0.5275	0.2115								

TABLE II.—*Continued.*

.	Time.	Quantity in cc.	Specific gravity.	Solids in 25 cc.	Ash in 25 cc.	Total 24 hours quantity.	Total daily solids.	Total daily ash.	% solids.	% ash.	Remarks.
91	8 A. M.–2 P. M.	183	1.010	0.4953	0.2006	600	12.5205	5.2298	2.09	.87	Medication, bile salts. 9 × 0.25.
	2 P. M.–8 P. M.	180	1.008¼	0.4936	0.2255						
	8 P. M.–2 A. M.	142	1.009	0.6293	0.2178						
	2 A. M.–8 A. M.	131	1.009½	0.6260	0.2186						
92	8 A. M.–2 P. M.	207	1.008¼	0.4791	0.2019	660	14.3758	5.6844	2.18	.86	" " 10 × 0.25.
	2 P. M.–8 P. M.	234	1.009¼	0.4832	0.2332						
	8 P. M.–2 A. M.	148	1.010¼	0.4978	0.2274						
	2 A. M.–8 A. M.	193	1.010¼	0.5329	0.1854						
93	8 A. M.–2 P. M.	212	1.010¼	0.5148	0.2020	787	15.9508	6.6657	2.03	.85	" " 11 × 0.25.
	2 P. M.–8 P. M.	246	1.009	0.5806	0.2055						
	8 P. M.–2 A. M.	102	1.008½	0.5408	0.2178						
	2 A. M.–8 A. M.	122	1.008¼	0.5258	0.1963						
94	8 A. M.–2 P. M.	164	1.008	0.4398	0.2276	634	13.3774	5.3684	2.11	.85	No medication.
	2 P. M.–8 P. M.	175	1.007	0.4126	0.2256						
	8 P. M.–2 A. M.	74	1.008¼	0.4681	0.2112						
	2 A. M.–8 A. M.	123	1.008½	0.4775	0.2101						
95	8 A. M.–2 P. M.	160	1.008	0.3921	0.2076	532	9.1367	4.5709	1.72	.86	" "
	2 P. M.–8 P. M.	172	1.008½	0.3918	0.2288						
	8 P. M.–2 A. M.	79	1.009½	0.4548	0.2222						
	2 A. M.–8 A. M.	110	1.010¼	0.4607	0.2187						
96	8 A. M.–2 P. M.	174	1.008¼	0.3785	0.2127	535	8.8009	4.7230	1.65	.88	" "
	2 P. M.–8 P. M.	169	1.007½	0.3898	0.2130						
	8 P. M.–2 A. M.	80	1.007½	0.4242	0.2167						
	2 A. M.–8 A. M.	100	1.008¼	0.4822	0.2107						
97	8 A. M.–2 P. M.	175	1.007¼	0.3827	0.2136	524	8.6022	4.4762	1.64	.85	" "

The 97 days over which our investigation extended may be subdivided into 13 different periods.

1st period	..... 3 days	.....	no medication.
2nd "	.....12 "	.....	medication, human bile.
3rd "	..... 4 "	.....	no medication.
4th "	..... 7 "	.....	medication, salol.
5th "	..... 2 "	.....	no medication.
6th "	..... 7 "	.....	medication, sublimate.
7th "	..... 5 "	.....	no medication.
8th "	..... 7 "	.....	medication, ox bile.
9th "	..... 5 "	.....	no medication.
10th "	..... 6 "	.....	medication, calomel.
11th "	.....18 "	.....	no medication.
12th "	.....17 "	.....	medication, bile salts.
13th "	..... 4 "	.....	no medication.
	97 days.		

After the first three days, when the normal flow of bile was observed, the patient began to take the pills made from her own bile. On the first day, the 4th day in the curve (Plate IX), she took three pills altogether, one at 2 P. M., one at 8 P. M., and the third one at 8 o'clock the following morning. The second day at the same hour two pills were given each time, making in all six pills in 24 hours; the 3rd day again 6 pills; the 4th day 3 x 3 pills; then 3 x 4, 3 x 5, and 3 x 6 pills each day. On the 8th, 9th, 10th and 11th day the patient was given at 2 P. M. and at 8 P. M. seven pills each time; and at 8 of the following morning always 6 pills, so that the total amount of pills given on any one of these four days was 20, corresponding to 500 cc. of fistulous bile. On the 12th day, under treatment with human bile—Jan. 26, 27—the patient had only 19 pills. On Jan. 27, at 8 A. M., she took the last pills of this sort.

During this period of observation—twelve days of medication with fistulous bile—the appetite of the patient, which was already good, showed a decided improvement. She objected no longer to eat butter and other fatty food, but on the contrary—as the nurse, who was ignorant of the nature of the medicine given, reported—she even asked for more butter. The weight of the patient was, on Jan. 15, the day bile was first given, 113½ lbs, a fortnight before it was 113

lbs. The patient was always weighed in a night-shirt, thin calico wrapper, stockings, cloth slippers, and with a bottle—the receiver for the bile. Two days after the bile pills were discontinued the patient weighed 119 lbs, a gain of 5½ lbs for a fortnight.

The fæces continued colored till January 22. The bulk of the fæces was the same as before. On the 23rd the patient had a smaller and softer movement of the bowels, with yellow color. On January 24th and 25th the patient had soft stools with brownish particles in it, which proved to be unabsorbed bile. On the 26th the patient had no movement of the bowels, and on the morning of January 27th, at the time when she took the last bile pills, she had a small hard stool of brownish yellow color.

The total amount of bile discharged through the fistula rose the first two days from 525 cc. to 545 and 586 cc., to fall again for the next two days to 529 cc. We had here a case similar to Copeman and Winston's: first a decided rise and later, in spite of continuing the medication, a drop in the flow of the bile to the normal. But as we increased the number of pills the flow of bile again increased and continued to do so, reading as highest mark 685 cc. on one day. With the increase in quantity secreted we found a qualitative improvement, the bile becoming more concentrated. The percentage of ash showed only a very slight gain during the whole time, as can be seen in the curve (Plate IX).

In the third period of observation—no medication again—we see a constant and rapid decrease in the flow of bile for the four days. The rapid falling off in quantity of bile is remarkable after withdrawal of the bile pills. It finds its analogy in the steady decrease of bile in animals after establishing a biliary fistula. Here, too, reabsorption of bile from the intestine being impossible, the discharge from the biliary fistula diminishes constantly and becomes more diluted.

In the case of our patient the quantity of bile decreased, and at the same time the quality became poorer, as fewer solids were contained in the secreted bile. The very slight rise in the percentage of ash in the secreted bile under medication with bile decreased as soon as the medication was stopped.

On the first day of this period the patient passed a small, slightly brownish colored, hard stool, of a size much smaller than usual when under no medication whatever. But the second day, after withdrawal of the bile pills, the patient passed again one of the excessively large and clay-colored stools, and she passed the same kind of stools the following days.

On the fifth day, after discontinuing the bile pills, we began the fourth period, using salol as the medicament. That time might not be lost, we did not wait till the flow of bile was again as high as normal. We felt ourselves justified in beginning this medication at such an early period, concluding that if salol was to be regarded really as a cholagogue, the results must be so plainly marked that no doubt would exist about their interpretation.

On the 20th day in the curve, that is from January 31 to Feb. 1, the patient took 1.2 grammes salol in four doses of 0.3 each. The drug was taken at 5 P. M., 1 A. M., 6.30 A. M. and 12 M. The next three days she was given four times a day 0.6 salol. On the last two days her urine assumed, on standing, the characteristic color of carbol urine, and the medication was therefore stopped. The patient felt perfectly well during the seven days she was given salol. Her weight had increased to 121 lbs on February 7, an increase of two pounds for the preceding two weeks.

Looking over the curve (Plate IX) we see on the third day of this period a considerable rise in the quantity of bile secreted. The quantity, which had dropped below normal, rose suddenly to normal, and even slightly above. During the whole period of administration of salol the biliary secretion remained slightly above normal. The percentage of solids increased with the rise in quantity, but the increase is not so pronounced as at the time the patient partook of the pills made from her own bile. The percentage of bile ash under salol showed a slight decrease.

Are the results obtained under salol medication to be looked upon as the effect of a cholagogue action of this drug? We are of the opinion that these results are not so conclusive as to allow a positive statement. The sudden rise in the flow of bile, on the third day,

could well have occurred normally, and is not necessarily to be attributed to the influence of salol. Similar variations in the flow of bile will be found later in the curve, when the patient was not under the influence of any drug.

The percentage of ash in the bile under the salol medication does not show any marked alteration as compared with the ash of so-called normal bile. The percentage of solids in the bile secreted in the last days under salol shows an increase. This fact, combined with the slight increase in total quantity of bile secreted, may speak for a cholagogue action of salol. But our knowledge of the normally occurring variations of all these factors is too limited to permit positive expressions based on the results obtained.

Although we do not wish to express a positive opinion as to the cholagogic or non-cholagogic effect of salol, we are of the opinion that our results at least prove one thing, that is, that bile secreted under salol is decidedly in the last four days of this period more concentrated than any other sample of bile which we collected during the whole course of our experiments, with the exception of bile secreted the two days after discontinuing salol and during the time the patient was taking either pills made from her own bile or ox bile or the mixture of pure biliary salts. The lauded action of salol as a peculiar cholagogue* which increases the flow of bile in quantity but diminishes its concentration, is decidedly, according to our results, not warranted.

The stools in the salol period did not show any difference from those passed when the patient was taking no medicine. The size was characteristically large, they were clay-colored, but the smell did not seem so offensive to us.

On the 27th day of observation the salol was discontinued, and for the next two days the patient was given no medicine. On the first day the flow of bile was even a trifle larger than on any day of the salol period. On the second day the quantity of bile secreted was slightly less than normal. The percentage of solids was on both days even slightly higher than on any day under the salol medication.

* Traité de Médecine. Publié sous la direction de MM. Charcot, Bouchard, Brissaud. Paris, 1892. Tome iii (A. Chauffard), pp. 681, 738.

On February 9, the 29th in the curve, the patient began to take sublimate in doses of 0.0015 gramme four times in 24 hours, that is to say at 2 P. M., 8 P. M., 2 A. M. and 8 A. M. This dosage of sublimate was kept up for the following four days. Afterwards the dose was increased to 0.003 four times a day for two days, so that the patient was under the sublimate treatment seven days altogether. After the second day of sublimate treatment the patient complained of neuralgic pains in the teeth and jaws, which, without any treatment, disappeared the following day.

During the time the patient was taking sublimate the flow of bile was, if anything, slightly less than normal. The slight increase of solids noticed in the salol period, and the two days following this time, had disappeared, and the percentage of solids became normal again. The percentage of ash remained practically unaltered. In the light of our results, sublimate has absolutely no stimulating effect upon the liver secretion; if it influences this secretion to any extent, it may very slightly diminish the total quantity of bile secreted instead of increasing its flow.

The patient was, when under the influence of sublimate, perfectly well, with the exception of the slight neuralgic pain on one day. Her appetite was, as usual, perfect. Her bowels moved regularly. The stools were clay-colored, but did not smell so bad as during the periods without medication. From February 16th until the 21st of the same month, the 36th to the 40th day on the curve (Plate IX), the patient was again without any medicine. The total amount of bile for six hours, from 2 A. M. to 8 A. M. on the 37th day, was not to be determined, as the patient had upset during her sleep the receiving graduate, leaving only enough in it to determine the specific gravity, the solids and the ash.

The amount of bile secreted during this period does not show any characteristic variations. It was, if anything, slightly greater than during the time the patient was taking sublimate. The percentage of solids and ash was normal. The weight of the patient on February 20 was 123 lbs—a gain of 2 lbs for the fortnight—a total increase of 9½ lbs from the beginning of our investigation.

On February 21, the 41st day in the curve, we began to give her pills made from ox bile, fel bovis inspissatus. The inspissated bile was first dried to a powder, and, with the aid of a little glycerin, pills were made, each pill corresponding to 0.5 dried ox bile.

On the first two days of this period she took three pills four times a day, corresponding to 6 grammes dried ox bile. The next four days the number of pills was increased to 4 x 4 pills, and on the last day of this period the patient had four times five pills, corresponding to 10 grammes dried ox bile. During the whole period the patient was in excellent health. There was no nausea, no diarrhœa—appetite good. The action of the heart showed no alteration whatever. Directly the first day that these pills were taken the flow of bile increased remarkably, from 521 cc. the day before to 694 cc. for 24 hours. This increase took place during the last 18 hours of the day, as the flow for the first 6 hours was not affected at all. With the increase of bile pills taken we observe a steady increase in the flow of bile. The largest quantity of bile was secreted on the day the patient took the greatest amount of bile pills. With the increase in the flow of bile the percentage of solids also rose. The amount of bile solids in the biliary secretion in our patient, being 1.6–1.7 per cent when under no medication, rose the second day under ox bile to 2.5 per cent, and remained about as high during the whole period. The percentage of ash of the bile secreted rose only slightly as compared with the bile of normal flow.

In spite of the comparatively large amount of bile given, the patient had during this period no loose movements of the bowels, whereas she did have, when taking her own bile, loose stools for two days. On the first two days the patient passed the ordinary very large clay-colored stools. On the third day the fæces were of a yellowish color, and on the fifth day they assumed a brownish color. During the time the patient was without bile the stools came in a large mass, which was almost a perfect cast of the rectum; but when under bile treatment the quantity of fæces diminished, and they appeared as small brown balls.

At the end of the 47th day the bile pills were stopped. During

the first 12 hours of the following day the patient still passed the same large amount of bile as when taking the bile pills, the secretion being very concentrated at the same time. From the 12th to the 18th hour of this day the secretion began to diminish, and during the last six hours of this day a decided decrease was unmistakable. With the diminution in the quantity a decrease in quality took place at the same time, as can be seen by the lower specific gravity. The quantity of bile which was secreted the last day under ox bile, being 770 cc., fell in the next 24 hours to 646 cc., and on the following day to 402 cc., the lowest quantity ever secreted during the whole time of our observation. On the third day after discontinuing the bile pills the secretion again rose to 496 cc., and then remained at about the same height the following days.

The first two days after the bile pills were stopped the fæces remained about the same in size and color as in the last days of the bile medication. On the third day very large and clay-colored stools were again passed. The difference in the size of the stools was so apparent that on seeing them one could tell whether the patient was taking bile pills or not.

For five days altogether, after withdrawal of the bile pills, the patient was without any medicine. On the fifth day of this period the bile of six hours was again spilled, so that the total quantity could not be ascertained; only the specific gravity, the solids and ash. But comparing in our table the results obtained for the different 6-hour periods of the last day with the corresponding periods of the previous day, the flow of bile had resumed again its normal condition, and we began to try the action of another supposed cholagogue, namely, calomel. The weight of the patient had increased in the meantime to 126 lbs, a gain of 3 lbs for the fortnight.

On the 53rd day in the curve (Plate IX), 4th–5th of March, the patient was given three times 0.03 gramme calomel in pills. The 54th day 4 times the same amount; the 55th day this was increased to 5 times, then to 6 and 7 times per day. On the 6th day under calomel she was given only 4 times 0.03 calomel. The medication was stopped, as the patient complained of soreness of the gums and

salivation, showing that mercury was absorbed in sufficient quantity. The fæces passed during this period—there was no aperient effect whatever of the mercury—were for the first 4 days of the characteristic large size and clay-colored. On the 5th day the color turned into greenish yellow, and on the 6th day they were brownish, covered with mucus and some drops of blood. With the exception of the complaints of the last day in this period the patient was perfectly well.

The flow of bile during the six days when calomel was administered shows, if anything can be inferred from such slight variations, that calomel decreased this secretion rather than increased it. Calomel, like sublimate, has no cholagogue effect. The percentage of solids and ash in the bile collected during calomel medication shows no difference from so-called normal bile.

We gave our patient calomel in small doses and not in large quantities as used sometimes by physicians when they wish to obtain the cholagogue action of this drug. That large doses of calomel have no cholagogue effect on a human subject with biliary fistula has already been demonstrated by Westphalen and Mayo Robson on their patients, and we did not see the necessity of repeating these experiments on our patient. We could dispense more easily with such experiments, as calomel, if not acting as a cholagogue in smaller doses given repeatedly, would decidedly be of less value for such purposes when given in quantities exhibiting an aperient effect.

The theory which has been advanced that calomel acts as a cholagogue by removing a small obstacle, such as mucous plugs, from the common duct need not be considered here, as such a supposed action would have nothing to do with a genuine cholagogue effect of the drug.

From the 59th day in the curve (Plate IX) until the 76th day the patient was given no medicine whatever. During the first days the patient still complained about soreness of the gums and passed fæces with some fresh and some old blood adherent. There was no diarrhœa, and her appetite was good. The bile secreted during this period did not show much variation from the normal flow. For about the first 8 days it was a little less than normal, but gradually increased

so that for the last six days of this period it averaged 518.5 cc. per day, practically the same as during the first three days at the beginning of our observation, when it was 514.3 cc. per day. During the last six days in this period the fæces did not contain any trace of blood, and were again clay-colored and of the large size noticed when the patient was taking no medicine. During the first six days of this period the size of the brownish fæces was smaller than usual when the patient was without medicine. The brownish color during this time is easily explained by the presence of blood in the fæces. Can the smaller size have been due to stimulation of the intestine by calomel, and consequently increased metabolism?

Considering the patient entirely recovered from the effects of calomel, there being no soreness of the gums, we began giving her a mixture of pure salts of glycocholic and taurocholic acid. The patient took the first day two pills twice, each containing 0.25 gramme of bile salts. The quantity of bile secreted rose for the corresponding 24 hours to 701 cc. from 533 cc. the day before. The bile secreted during the first six hours of this day did not show any difference as compared with the bile secreted during the corresponding time of the previous day. In the second six hours the flow of bile was already much increased, the specific gravity was higher and the color a dark green. Qualitative examination of this bile showed a large amount of albumin present. The same result was found in the bile secreted the following twelve hours. As the patient felt perfectly well and the fistula did not show any change, the presence of the large amount of albumin in the bile could not be accounted for otherwise than by the assumption that the amount of bile salts given at a time was too large, or that the absorption was too rapid. The single dose was therefore reduced to one pill, whereas the total dose *per diem* was not reduced, the patient taking every six hours one pill. On the following day the quantity of bile was somewhat reduced, as also the amount of albumin in it. On the 4th day of this period the biliary secretion showed only a trace of albumin, which was always present, and was probably the result of the wound. But in spite of this we did not increase the amount of bile salts until the beginning of the 11th day,

so that the patient had, from the 76th day until the end of the 86th day in the curve, only one gramme of bile salts each day. From the 87th to the 93rd day the patient was given every day one more pill of the bile salts, so that she had on the 87th day 5 pills, on the 88th 6 pills, on the 89th 7 pills, on the 90th 8 pills, on the 91st 9 pills, on the 92nd 10 pills, and on the 93rd day 11 pills. One pill was always given at a time, intervals of at least one hour intervening. Even with this increased amount of bile salts the secretion did not show any more albumin. The patient felt perfectly well the whole time.

If we try to explain the results obtained in this period we must of course leave out, in our consideration, the results obtained for the first three days, when the quantity of bile discharged was very high, but contained a large amount of albumin.

As we see on the curve (Plate IX) the quantity of bile secreted, when the patient was taking bile salts, was also considerably increased. As long as the patient was taking only one gramme of bile salts per day the increase in the daily quantity was not so marked, and the percentage of bile solids did not increase. As soon as the quantity of bile salts given was increased the percentage of bile solids was proportionately increased, and the quantity of the bile also rose. On the day the patient was taking the largest number of bile-salt pills the biliary secretion was the greatest ever seen in our patient, rising to 787 cc. The percentage of solids in the secretion did not reach such a high point as under the treatment with ox bile. The percentage of ash in the period of bile salts was less than in the time the patient was taking pills made either from her own bile or from ox bile.

The fæces passed during the whole period of bile salts, with the exception of the movement of the last day of this time, had been typically clay-colored and did not seem to be influenced in the least by the bile salts. Only on the last day the patient had a movement of brownish fæces formed into smaller balls, such as were observed when she was taking a larger amount of pills made from dried bile.

The most probable explanation of the results obtained with bile salts is that they are probably the substances in the bile which have the most powerful cholagogue effect, and being easily soluble in water,

they are for the most part absorbed in the upper part of the digestive tract—stomach (?) and small intestines—and so reaching the liver quickly, may stimulate this organ more powerfully than dried bile would do. This stimulation may lead even to a pathological secretion of the liver, as shown by the presence of a large amount of albumin in the secretion of the first days, when patient was taking the pills made from bile salts. This over-stimulation can be avoided if bile salts are given in repeated smaller doses. That dried bile, even in larger quantities, has not this undesirable effect may be explained by the circumstance that on account of the mucin, etc., present, a reabsorption of these pills is not so quickly accomplished, and therefore the action is more delayed and gradual. If this explanation should be the right one, pure bile salts, if given in a vehicle which delays absorption, such as a mucilaginous mixture, may prove in action identical with dried bile.

At 2 o'clock P. M. of the 93rd day the patient was given the last pill of bile salts. The biliary secretion in the following six hours amounted to 246 cc., the highest quantity observed during any six hours while the patient was under our observation. Afterwards the secretion began to decrease rapidly, so that at the end of the first 24 hours after withdrawal of the bile salts the total secretion amounted to 634 cc., as against 787 cc. the previous 24 hours. On the second day the amount fell to 532 cc. for 24 hours, and the following two days was 535 cc. and 524 cc. With the decrease in the secretion we observe, just as after the period when the patient was taking the pills made from her own bile, a rapid diminution in the percentage of solids, whereas the percentage of ash was not altered. In the last three days of our observation, the patient being under no medication whatever, just as in the first three days, the average amount of bile secreted per 24 hours amounted to 530 cc., whereas in the first three days of our observation, three months previously, the average was 514.3 cc. bile per day. The percentage of solids and ash was at both times also practically the same.

On the second day after the pills of bile salts were stopped the patient had a movement of the bowels of which one part was identical

with the one passed on the last day when taking bile salts; that is to say, the fæces were brownish colored and formed into small balls. The other half of the same movement was clay-colored and of the usual large size. On the 96th and 97th day of our observation the patient passed again exactly the same kind of large clay-colored stools as in the beginning, the only difference being that the odor at the end was not so offensive.

The patient's weight, which was 113½ lbs in January, had increased to 127½ lbs, a gain of 14 lbs in 97 days. Her general health was very good, and she left the hospital,* a few hours after the last amount of bile was collected, in the best of spirits, in spite of the inconvenience of a biliary fistula.

If we review the results of our experiments we must say that of the drugs investigated we got only a decided increase in the quantity of bile, and at the same time a higher percentage of solids, with human bile, with ox bile, and with the mixture of pure bile salts. The results with salol are not decisive. The quantity of bile discharged through the fistula when the patient was taking salol did not vary so much from the normal that we could be sure that the effect was due to the action of the drug. Nevertheless the percentage of bile solids under salol treatment showed an increase when compared with bile of normal flow.

Under corrosive sublimate or calomel treatment the quantity of bile discharged through the fistula was, if anything, less than normal, whereas the percentage of solids did not vary from the normal.

The results obtained with bile or its characteristic salts show an increase in the quantity of bile and a higher percentage of its solids. This does not prove that this increase in the percentage of solids is due to an absolute increase of characteristic bile constituents. To see whether the characteristic bile salts had increased with the quantity of bile, a quantitative estimation had to be made.

* Since leaving the hospital the patient has come to the laboratory every two weeks regularly. She has felt perfectly well during the summer with the exception of several supposed malarial attacks. Her weight, which had increased the first week to 128½ lbs, dropped to 125 lbs during her malarial attacks. Her weight during the last four weeks was 130 lbs, it now being six months since her discharge from the hospital.

We determined the quantity of glycocholic and taurocholic acid in bile collected during different periods.* The methods of quantitative estimation of biliary salts are not absolutely accurate, but the estimations are sufficiently reliable to permit conclusions when the results obtained show a marked difference in the percentage of these salts. In the mixture of pure bile salts obtained by the quantitative estimation, we determined the quantity of sulphur so as to be able to see how much taurocholic acid and how much glycocholic acid were present.

But as the quantitative estimation of bile salts in bile does not give absolutely reliable results (the precipitated mixture of bile salts is not chemically pure, and therefore the quantity of sulphur varies), we estimated the sulphur also directly in the dried bile of different periods to determine more accurately the amount of taurocholic acid.

The only other chemical substances in our bile containing sulphur were sulphates, which were so small in amount that they practically could not be estimated.†

The method of estimation of the bile salts was as follows: Bile dried to constant weight at 110° C. was treated with boiling absolute alcohol (Squibb's) to exhaustion, that is to say until a cooled and filtered portion of the alcoholic solution gave no precipitate with absolute ether (Squibb's). The combined alcoholic solutions were allowed to stand for 24 to 48 hours, depositing a small quantity of a fine amorphous substance. They were afterwards filtered, evaporated and dried at 110° C., and then taken up with a small quantity of absolute alcohol, a slight residue remaining. The alcoholic solution was evaporated to a smaller bulk, cooled and precipitated with a large

* Owing to the fault of the laboratory attendant the solid residues of the bile excreted by the patient under salol and corrosive sublimate have been mislaid, and in these cases, therefore, a quantitative estimation of the bile salts has been impossible.

† O. Hammarsten's "Zur Kenntniss der Lebergalle des Menschen" (loc. cit.) was not known to me (F. P.) until I had sent this article for publication. We therefore did not test the bile of our patient for Hammarsten's ether-sulphonic acid. We have since, with the bile salts of five different periods, which remained at our disposal, tried Hammarsten's process for ether-sulphonic acid and found it to be entirely absent.

excess of absolute ether. The alcoholic-ethereal solution was allowed to stand over night and the supernatant liquid decanted. The residue—the bile salts—were washed with ether dried at 110° C. and weighed.

The bile collected each day, with the exception of the quantity necessary for the quantitative estimation of solids and ash, was evaporated on the water-bath, and the residues of the different days belonging to one period were thoroughly mixed together and this mixed bile residue analyzed. In the following we give the results of the analyses of bile residue of different periods.

I. Analyses of bile residue from the first period, January 12–13 to 14–15, when the patient was under no medication:

1) 5.0000 bile residue gave 1.2328 bile salts = 24.66% bile salts.
2) 4.9700 " " " 1.2335 " = 24.83 "
3) 3.4022 " " " 0.7322 " = 21.52 "

Estimation of sulphur in the bile salts obtained in analysis:

1) Taken 0.3317 bile salts gave 0.0221 $BaSO_4$ = 0.91% S.
2) " 1.0240 " " 0.0723 " = 0.97 "

Estimation of sulphur contained in dried bile:

a) 1.6698 bile gave 0.0480 $BaSO_4$ = 0.39% S.
b) 1.5920 " " 0.0447 " = 0.39 "

Calculated (mean of the two sulphur estimations in bile), taurocholate of sodium = 6.57%.

II. Analyses of bile residue when patient was taking pills made from her own bile. Period II, January 15–16 to January 26–27:

1) 3.8205 bile residue gave 0.9505 bile salts = 24.88% bile salts.
2) 4.6058 " " " 1.0507 " = 22.80 "

Estimation of sulphur contained in dried bile:

a) 4.5993 bile gave 0.1751 $BaSO_4$ = 0.52% S.
b) 5.0142 " " 0.1915 " = 0.52 "

Calculated (mean of the two sulphur estimations in bile), taurocholate of sodium = 8.78%.

III. Analyses of bile residue when the patient was taking pills made from ox bile. February 21–22 to February 27–28:

1) 5.0463 bile gave 2.2249 bile salts = 44.09% bile salts.
2) 4.4559 " " 1.9796 " = 44.43 "
3) 3.4123 " " 1.5864 " = 46.49 "

Estimation of sulphur in the bile salts obtained in analysis:

1) Taken 1.8815 bile salts gave 0.2818 $BaSO_4$ = 2.06% S.

Estimation of sulphur contained in dried bile:

a) 3.2157 bile gave 0.2855 $BaSO_4$ = 1.22% S.
b) 2.6966 " " 0.2382 " = 1.21 "
c) 3.5682 " " 0.3397 " = 1.31 "

Calculated (mean of the three sulphur estimations in bile), taurocholate of sodium = 20.91%.

IV. Analyses of bile residue when the patient was taking calomel. March 4–5 to March 9–10:

1) 5.0000 bile gave 1.2718 bile salts = 25.44% bile salts.
2) 2.4985 " " 0.6069 " = 24.29 "

Estimation of sulphur in the bile salts obtained in analysis:

1) Taken 0.8416 bile salts gave 0.058 $BaSO_4$ = 0.95% S.
2) " 0.6639 " " 0.0369 " = 0.84 "

Estimation of sulphur contained in dried bile:

a) 2.3815 bile gave 0.1075 $BaSO_4$ = 0.62% S.
b) 1.8018 " " 0.0623 " = 0.48 "

Calculated (mean of the two sulphur estimations in bile), taurocholate of sodium = 9.18%.

V. Analyses of bile residue when the patient was taking no medicine, eight days after calomel had been discontinued. March 18–19 to March 27–28:

1) 5.0000 bile residue gave 1.1087 bile salts = 22.17% bile salts.
2) 5.6835 " " " 1.3821 " = 24.32 "

Estimation of sulphur in the bile salts obtained in analysis:

1) Taken 0.3420 bile salts gave 0.0235 $BaSO_4$ = 0.94% S.
2) " 0.9090 " " 0.0605 " = 0.91 "

Estimation of sulphur contained in dried bile:

a) 2.5098 bile gave 0.0748 $BaSO_4$ = 0.41% S.
b) 1.5690 " " 0.0462 " = 0.40 "

Calculated (mean of the two sulphur estimations in bile), taurocholate of sodium = 6.82%.

VI. Analyses of bile residue when patient was taking pills made from the mixture of glycocholate and taurocholate of sodium. April 5–6 to April 14–15. The bile residue of the first eight days of

this period was not analyzed, as the excretion showed for the first three days a large amount of albumin.

1) 4.6182 bile residue gave 1.5297 bile salts = 33.12% bile salts.
2) 8.0675 " " " 2.8519 " = 35.35 "
3) 10.1160 " " " 3.5482 " = 35.08 "

Estimation of sulphur in the bile salts obtained in analysis:

1) Taken 1.1682 bile salts gave 0.2247 $BaSO_4$ = 2.64% S.

Estimation of sulphur contained in dried bile:

a) 4.0970 bile gave 0.2988 $BaSO_4$ = 1.00% S.
b) 3.5310 " " 0.2925 " = 1.14 "
c) 3.7725 " " 0.2931 " = 1.07 "

Calculated (mean of the three sulphur estimations in bile), taurocholate of sodium = 17.92%.

We append here the analyses of the dried ox bile and of the mixture of bile salts from which pills were made for the patient.

Analyses of dried ox bile. This sample was used to make the pills of dried ox bile:

1) 2.8058 ox bile gave 0.3218 ash = 11.11% ash.
2) 3.3381 " " 0.3857 " = 11.55 "

1) 4.8449 ox bile gave 4.0466 bile salts = 83.52% bile salts.
2) 2.5914 " " 2.1278 " = 82.11 "

Estimation of sulphur in the bile salts obtained in analysis:

1) Taken 1.1765 bile salts gave 0.2702 $BaSO_4$ = 3.15% S.

Estimation of sulphur contained in dried ox bile:

a) 6.2205 ox bile gave 1.1108 $BaSO_4$ = 2.45% S.
b) 3.0900 " " 0.6302 " = 2.80 "
c) 3.0390 " " 0.5882 " = 2.66 "
d) 3.6515 " " 0.6794 " = 2.56 "

Calculated (mean of the four sulphur estimations in ox bile), taurocholate of sodium = 43.92 %.

Analyses of the bile salts prepared from ox bile:

1) 0.9160 bile salts gave 0.2829 $BaSO_4$ = 4.24% S.
2) 0.9803 " " 0.3039 " = 4.26 "

Calculated (mean of the two sulphur estimations), taurocholate of sodium = 71.30%.

In Table III will be found the results of the analyses of the bile residues of different periods. The percentage of taurocholate of sodium is calculated from the quantitative estimation of sulphur in the dried bile, and not from the sulphur determined in the mixture of bile salts obtained in their quantitative estimation. We do not give the glycocholate of sodium in percentage (to be calculated by subtraction of taurocholate from the total quantity of bile salts), but we wish it to be understood that we do not lay any special stress on taurocholate alone.

TABLE III.

Bile residue contains	Bile salts in per cent.			Mean.	Taurocholate sodium in per cent.
Period I. No medication.	24.66	24.83	21.52	23.67	6.57
Period XI. No medication (after calomel discontinued)	24.32	22.17		23.25	6.82
Period II. Medication (pills from human bile).....	24.88	22.80		23.84	8.78
Period X. Medication (calomel) ..	25.44	24.29		24.86	9.18
Period XII. Medication (bile salts)	33.12	35.35	35.08	34.52	17.92
Period VII. Medication (ox bile)..	44.09	44.43	46.49	45.00	20.91

The quantity of bile salts in the bile residue collected during the first period of our observation, when the patient was taking no medicine, was on the average 23.67 per cent. The quantity of taurocholate of sodium in it amounted to 6.57 per cent. The bile residue in the eleventh period, that is, over two months later, when the patient was again under no medication, contained the same percentage of bile salts (23.25 per cent) with the same amount of taurocholate of sodium (6.82 per cent). The quantity of bile secreted at both periods was also practically the same, as also the percentage of solids. In the period of medication with human bile, the bile residue contained also 23.84 per cent of bile salts, but these were made up by 8.78 per cent taurocholate of sodium as corresponding to 6.57 and 6.82 per cent found in so-called normal bile. The percentage of bile salts was therefore seemingly not influenced by the medication with human bile. How far this result was influenced by the fact that on the two days of this period, just when she was taking a large number of pills,

the patient was having loose stools and was losing unabsorbed bile with the fæces, cannot be stated. The increase in the percentage of taurocholate of sodium in the bile residue, when the patient was taking pills made from her own bile, is curious, and we fail to find a proper explanation of it. Of course we must not forget that we do not know anything of the normally occurring variations in the relation of glycocholic and taurocholic acid in bile, and that such a difference as found above may be a normally occurring variation.

In spite of the fact that the percentage of bile salts in the biliary residue of this period showed no rise, the total amount of bile salts in this period is a much larger one than in the period when the patient was taking no medicine or even when taking calomel. The total amount is much larger on account of the higher percentage of solids found in the bile when the patient was taking pills made from her own bile and because of the larger quantity of bile secreted in this period. We got not only more bile discharged per day, but at the same time the percentage of biliary solids was higher, and as the percentage of bile salts remained the same, this gives an increase in the absolute quantity of bile salts from two sources. Or in other words, human bile given internally increased the quantity of bile discharged through the fistula, and the absolute amount of bile salts was relatively and absolutely increased at the same time.

The residue of the bile collected under medication with calomel showed 24.86 per cent of bile salts, of which 9.18 per cent were taurocholate of sodium. This would mean an increase of about 1 per cent of bile salts in the residue. But on account of the inaccuracy of quantitative estimation of bile salts, we cannot lay much stress upon this small increase in the percentage. The estimation of taurocholate of sodium by direct determination of sulphur in the bile residue being nearly absolutely correct, gave, as already stated, 9.18 per cent of taurocholate of sodium, that is, an increase of more than 2 per cent over that found in the bile residue of so-called normal bile. In the absence of any real knowledge of the normal composition of bile and of its normal variations, a satisfactory interpretation of these facts is far from easy, and we must be contented for the present with unsatis-

factory surmises. One view that might be taken is that calomel influences the metabolism directly, and thus the composition of bile indirectly. As we have seen, the quantity of bile when the patient was taking calomel did not increase, nor was there an increase in the percentage of solids. The slight increase of 1 per cent in the amount of bile salts may, on account of the inaccuracy of the quantitative estimation of bile salts, be only apparent and not actual; but even if this increase in the percentage of bile salts were positive, the total amount of bile salts secreted under calomel would not be increased, as with the same percentage of solids the quantity of bile discharged in the time units was rather less than normal when the patient was taking calomel. There is, therefore, no real cholagogue effect to be attributed to calomel, and we think that the curious change in the proportion of taurocholate of sodium is best explained for the present by a direct action of calomel on metabolism.

The results obtained when the patient was taking pills made from bile salts or from dried bile are so striking that their interpretation is an easy one. In both periods we got, as will be remembered, a large increase in the quantity of bile secreted and an increase in the percentage of solids. The bile residue under treatment with biliary salts showed 34.52 per cent of bile salts, of which 17.92 per cent was taurocholate of sodium, whereas the bile residue under the treatment with dried bile gave 45 per cent of bile salts, of which 20.91 per cent was taurocholate of sodium. The bile residue of so-called normal bile had only 23.67 (23.25) per cent bile salts, of which 6.57 (6.82) per cent was taurocholate of sodium. We had therefore in the two periods in which the patient was taking pills made either from bile salts or from dried ox bile, not only much more bile with a higher percentage of solids, but also the bile residue contained a much higher percentage of bile salts. The characteristic salts in the bile residue under treatment with dried ox bile nearly doubled in percentage. The much larger increase of bile salts in the bile residue when the patient was taking pills made from dried ox bile is to be accounted for by the absolutely larger quantity of bile salts taken by the patient when she was under this treatment as compared with the quantity of bile

salts taken when she was given pills made from bile salts. The difference in the proportion of taurocholate of sodium to the total amount of bile salts found in the bile residue of these two periods is also easily explained by the difference in the proportion of taurocholate of sodium in the different pills. The pills of bile salts contained relatively more taurocholic acid than the pills made from dried ox bile, and the bile residue in the bile salt period contained relatively more taurocholate than in the period when the patient was taking pills made from dried ox bile.

The quantitative estimation of bile salts in the bile residue of these two periods established with absolute certainty the fact that the increase in the percentage of solids of the bile secreted during these times was due to an increase of characteristic bile salts in the bile. There can no longer be any doubt that bile or its characteristic bile salts, after absorption, increase the quantity of bile secreted, and also the relative and absolute amount of characteristic bile salts contained in this secretion.

In the description of the different periods we have mentioned a change in the size and the color of the fæces when the patient was taking dried bile. That the large size of the stools was due to the large amount of unabsorbed fat which they contained was evident. The smaller size of the stools when the patient partook of a larger amount of bile pills was probably due to a decrease of fatty substances present. To study this question, exact determination of the metabolism of our patient would have been necessary. But during the time the patient was in the hospital the possibility of such a research was not given. Nevertheless we analyzed the dried fæces of the patient on 17 different days scattered over different periods. The results obtained speak in favor of the above supposition, as we found differences in the percentage of fat varying between 68.30 and 43.37 per cent of the dried fæces. We do not publish the results of these 17 analyses, as they cannot prove accurately the influence of bile given *per os* on digestion, as we did not know the amount of food taken. We hope in the near future to publish results of the study of this subject.

In concluding this communication we wish to make a few remarks as to the form and the therapeutical application of ox bile. From the results of our investigation it seems best to use the crude ox bile, fel bovis insp., and to make the dried substance into pills. The purified ox bile could easily act as pure bile salts did in the beginning of the bile-salt period.

The continued influence of bile on the digestive process in the stomach is not known—laboratory experiments with artificial digestion cannot be relied upon, as they do not imitate natural conditions—and it seems to us to be wise, therefore, to use salol as a coating for ox bile pills so as to prevent any continued action of bile already in the stomach.

The quantity of ox bile to be used for medicinal purposes need of course generally not be so large as in our experiments where no bile whatever reached the duodenum by the natural way. The practical experience which one of us had in the last seven months seems to show that 0.25 gramme dried ox bile made up into a pill is of a convenient size. Two such pills given three times a day, or at the most five such pills three times a day, seemed to be the quantity required for practical purposes. To begin with a small number of pills, and if necessary to increase the number gradually, and then regulate the amount to be taken according to their effect, seems to work well.

The rational indication for the uses of ox bile is of course not to be given at present, as our knowledge of the uses of bile in the economy is only scanty, and still less well understood are the cases where bile is wanting.

For the present we will only say that bile may be useful in those cases where so-called cholagogues are now prescribed as well as in certain cases of constipation and possibly in cases where we wish to increase absorption of fat. Further clinical experience is necessary to settle these questions and to determine the proper place which bile should hold in therapeutics.*

* Bile was used, and highly valued therapeutically, centuries ago. Boerhaave relates having used, with marked success, the gall of the eel and also that of the pike. Charles Clay, a physician in Manchester, England, pub-

lished a paper "On the Exhibition of Inspissated Ox Gall in Various Diseases" (*The Medico-Chirurgical Review and Journal of Practical Medicine*, London, 1842, p. 278). The above statement regarding Boerhaave is quoted from this. Clay was led to try bile as a medicinal agent by a remark of Dr. Peacock (*Lancet*, vol. i, 1836-1837, p. 398), that the pains in cancerous breasts were greatly alleviated when the system exhibited an accumulation of bile (jaundice and white stools). Clay therefore tried ox gall in cancerous breasts, but with varying results. But he relates very good results with ox bile, in cases of constipation, especially constipation due to lead poisoning.

Later on, bile as a medicine fell into disuse. During the last decade a few other papers relating to the medicinal value of bile have been published, but they were based principally on clinical observation or were the result of mere speculation.

That these papers did not tend to advance the medicinal use of bile is proven by the fact that until a few years ago bile was not officinal in the pharmacopœias of Europe or the United States. In the last edition of the U. S. Pharmacopœia bile again found a place. The United States Dispensatory of 1894 has only the following words about the medicinal properties and uses of bile: "Bile was formerly highly valued as a remedy in numerous complaints, and was considered peculiarly applicable to cases attended with deficient biliary secretion. It is supposed to be tonic and laxative."

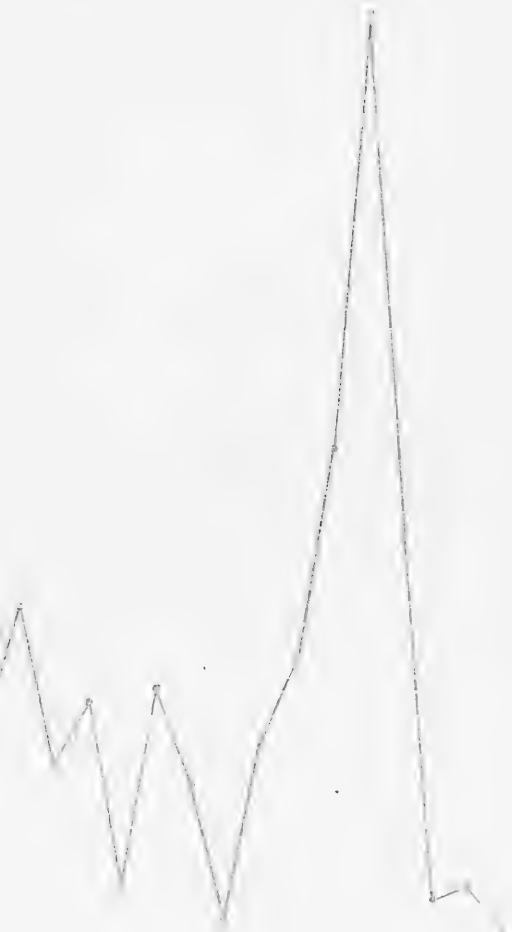
T. Mitchell Bruce (Materia Medica and Therapeutics, London, 1892, p. 410) says of bile: "It is a bitter and cholagogue purgative of doubtful value."

In German-speaking countries bile is thought to be of still less value, as may be gathered from a note in the well-known book of H. Nothnagel and M. T. Rossbach (Handbuch der Arzneimittellehre, 17te Auflage, Berlin, 1894, p. 640). The authors say: "Thereapeutically bile is to be absolutely rejected. There is no condition in which its usefulness can be proved."

DESCRIPTION OF PLATE IX.

Curves representing graphically results recorded in detail in Table II, pp. 72–83.

700
640
600
580
400



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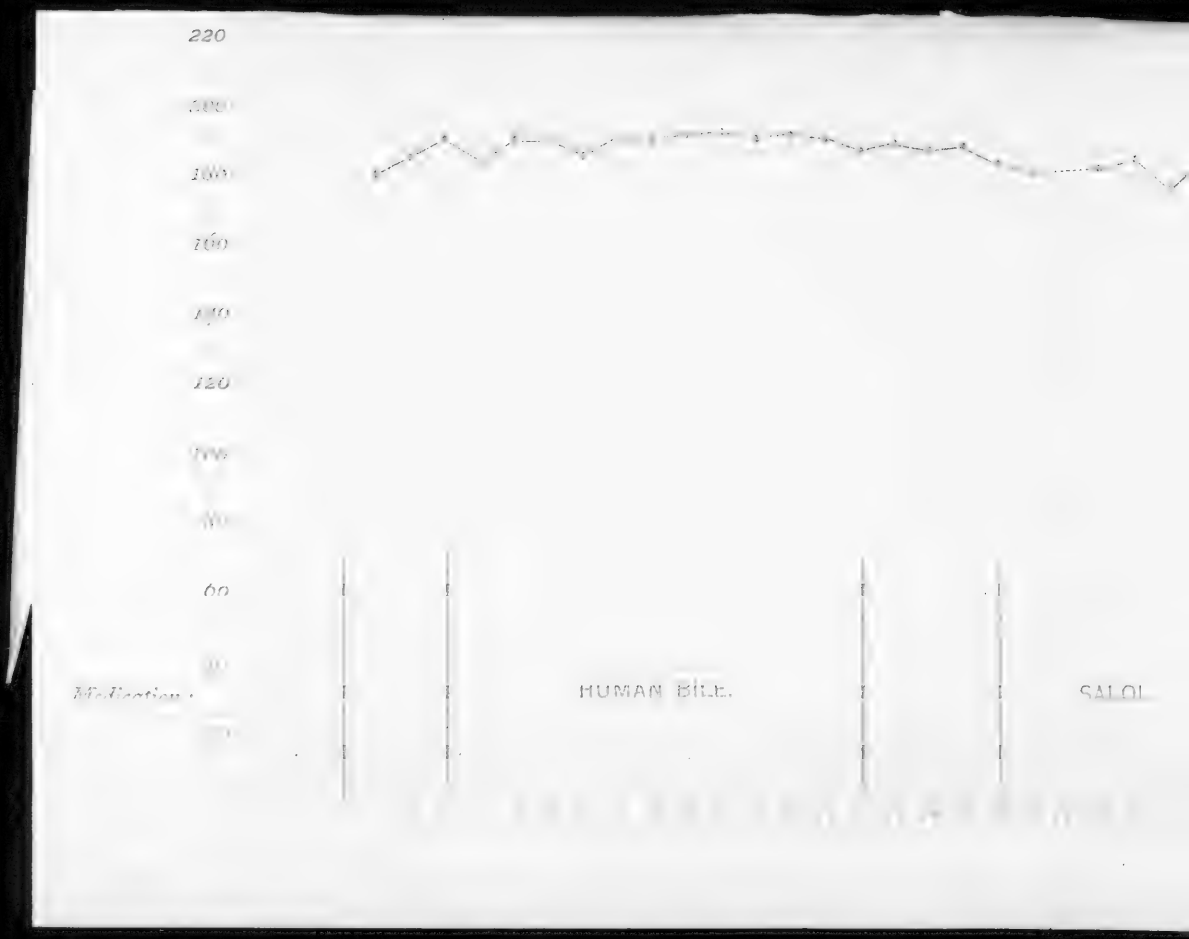
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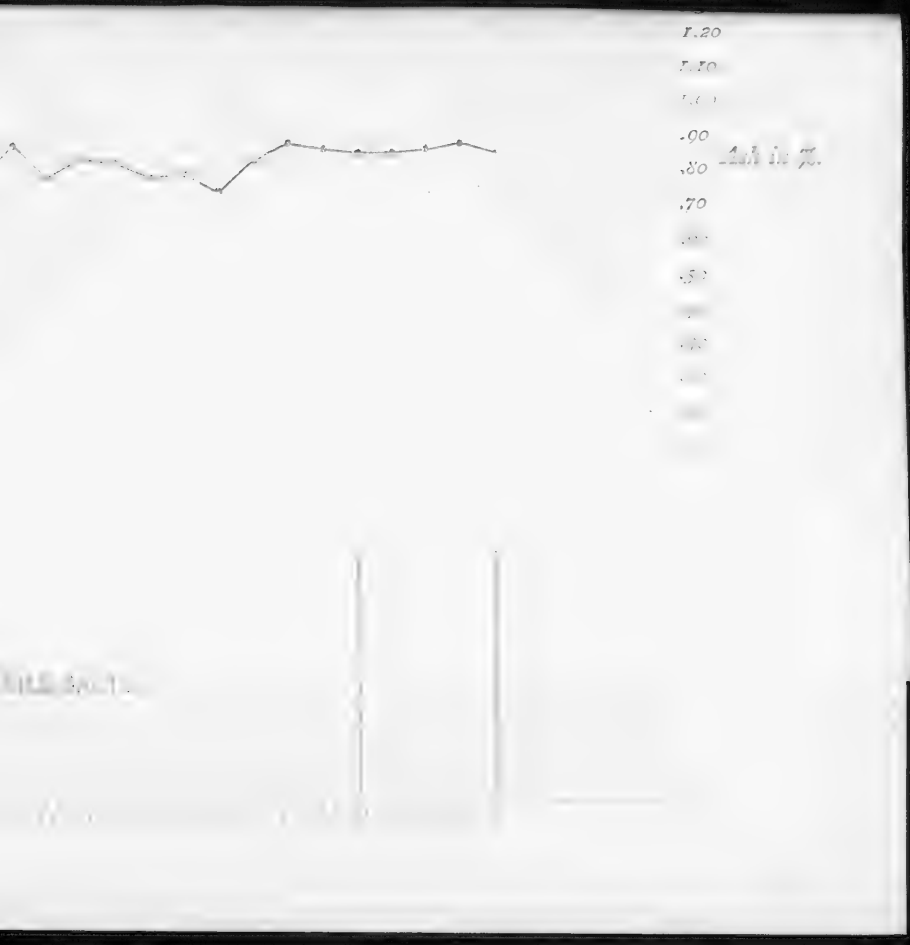
Medication
HUMAN BILE.
SALOL


1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
Ash in %.

THE INFLUENCE OF PHLORIDZIN ON THE BILE AND LYMPH.

By PHOEBUS A. LEVENE, M. D.

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In my article on Phloridzin Glycosuria, published in 1894,* I had in view the demonstration of two propositions: (1) That there existed no sufficient grounds for v. Noorden's assumption that phloridzin glycosuria does not at all resemble any forms of spontaneous diabetes mellitus, and that, therefore, the mechanism of this experimental glycosuria cannot be applied to the explanation of any clinical forms of diabetes; and (2) that the appearance of sugar in the urine in this experimental glycosuria could not be satisfactorily explained by mere elimination from the blood.

Several facts had led me to doubt the "elimination theory." In the first place v. Mering himself, who examined the blood for sugar only after the injection of phloridzin, found that the amount of sugar did not differ much from that which occurs very often in normal dogs, and therefore could not be called subnormal. Again, the work of Minkowski proved that after the extirpation of the kidneys and subsequent phloridzin injection there was in fact some slight increase of sugar in the blood. Lastly, Cornevin found an increase of sugar in the milk after phloridzin injection. To these facts I was able to add from my own experiments that the venous blood of the kidneys never contained less sugar than the arterial, and that the amount of sugar in the whole kidney after the phloridzin injection was higher than normal.

Very recently Zuntz, again recurring to the study of "phloridzin diabetes," has shown in a very ingenious way that the appearance of

* References to the literature are at the end of this article.

sugar in the urine after phloridzin injection may be due exclusively to the activity of the kidneys. This investigator injected very small quantities of phloridzin directly into the renal artery and afterward collected the urine of each ureter separately. He found that the urine of the kidney into which phloridzin had been injected contained sugar almost immediately, whereas in the other it was demonstrable only after some time, and then in much smaller quantities. Zuntz attempted further to limit the action of phloridzin to one kidney only, and therefore ligated the renal veins of the side upon which the glucoside was injected. But immediately after ligation the secretion of urine of the corresponding kidney was suppressed and sugar at once appeared in the urine of the other kidney, where without ligation it was usually demonstrable only after some time.

In these later experiments Zuntz sees a new corroboration of the elimination theory of v. Mering and a new disproof of the supposition expressed in my previous article.

Zuntz says:

"Verfasser hat die Nierenvene behufs Einführung einer Canüle abgeklemmt. Darnach aber hört die Urinsecretion für längere Zeit auf, es kann also auch keine Abnahme des Zuckergehaltes in der Vene erwartet werden. Noch weniger berechtigt ist Levene aus dem erhöhten Zuckergehalt der Niere selbst auf eine Beziehung derselben zur Zuckerbildung zu schliessen. Unvermeidlich findet sich in dem Canalsystem der Niere eine gewisse Menge Urin; da dieser Urin nach Phloridzinzufuhr 10 pro cent und mehr Zucker enthält, muss die Zuckerbestimmung in der gesammten Niere einen höheren Gehalt ergeben, als bei normalen Thieren, ohne dass man daraus folgern darf, der Zucker sei im Nierenparenchym gebildet worden."

But, if the suppression of the secretion of the urine immediately after stagnation actually takes place, this fact by itself does not by any means show that all the functions of the renal epithelium are suppressed at the same time, and there is no reason why the epithelium, stimulated or influenced in some way by the glucoside, could not for a time at any rate continue to absorb sugar from the blood.

Beyond doubt Zuntz's experiment affords a very striking argument

in favor of the supposition that the appearance of sugar in the urine after administration of phloridzin can be produced by the kidneys alone. Nevertheless by itself it proves nothing more, and fails utterly to explain the way in which the kidneys produce this effect. The latter might just as well be due to mere elimination as to local overproduction of sugar in the kidney. Important, therefore, as is Zuntz's discovery in showing the possibility of the occurrence of renal diabetes, it does not add much to the previous facts at our command for the explanation of the mechanism of phloridzin glycosuria.

The fact discovered by Zuntz, that the time which elapses between the injection of phloridzin directly into the arteries of the kidneys and the appearance of sugar in the urine is so short, induced me to attempt to apply the same method to another secretory organ, in order to see whether or not it would respond to the stimulus in the same way as the kidneys. As I have stated before, there is some mention in the literature that the mammary glands resemble the kidneys in this respect.

I chose for this purpose the liver. The experiments were conducted in the following way: The animals were narcotized by means of ether. After the gall bladder had been emptied of its contents a cannula was inserted into it and a few cubic centimetres of a 0.5 per cent solution of phloridzin in alkaline water were injected directly into the portal vein. The specimens of bile collected before and at different times after the injection were separately treated with 10 volumes of 95 per cent alcohol for 24 hours and filtered. The filtrate was decolorized by means of bone-black, evaporated to dryness, dissolved in water, filtered, and the resulting filtrate was tested with Fehling's solution. The experiment was repeated on five dogs. In two of them the blood-vessels of the kidneys were ligated previous to the injection of phloridzin. In all cases a very distinct reduction of the copper appeared with bile collected after the injection. In some cases a slight reduction took place also in the normal bile; this was probably due to the effects of the anæsthetic, and was never so great as in the other cases. As the amount of sugar in the bile was not quite sufficient for a quantitative examination, I decided to observe the influence of

phloridzin injection on animals with biliary fistulæ, where I could collect pure bile without inconvenience to the animal and hence without the use of narcotics. I performed this operation on two dogs with the following results:

Experiment, February 17, 1896.—Dog; weight, 20 kilos; operation for biliary fistula performed under anæsthesia. The cannula was kept open till the following morning, when it was closed. February 18, 11 A. M.: the normal bile was collected and a solution of 2.5 grammes of phloridzin in alkaline water was injected hypodermically. At 3 P. M. 25 cubic centimetres of bile were collected. At 6 P. M. 19 cubic centimetres of bile were collected. The cannula was left open till the following morning, when it was again closed for a few hours and the bile collected. These operations were repeated until February 21, when the dog was killed, as a slight suppuration had taken place around the wound. All the specimens of bile were treated in the above-mentioned way. Reduction of the copper solution took place in all after the injection of phloridzin, but did not appear in the normal bile. On February 21 the urine still possessed high reducing power.

Experiment, March 24, 1896.—Dog; weight, 9 kilos; operation for biliary fistula performed under anæsthesia; the fistula was left open until the following morning. March 25, 10 cubic centimetres of normal bile were obtained and a solution containing 2 grammes of phloridzin was given to the dog by the mouth; a portion was vomited. At 3 P. M. 7 cubic centimetres of bile were collected. As the quantity of bile secreted thereafter was very small, the dog was killed. The bile obtained before the injection of phloridzin possessed no reducing power, that after the injection showed a distinct reduction.

Though it is worthy of note that the reducing power of the bile was never so high as that of the urine, it must be remembered that the question for me was whether phloridzin acts exclusively on the kidneys or not. The foregoing experiments, as well as those of Cornevin, tend to answer the question in the negative. On the other hand these facts alone do not exclude the elimination theory.

In order to obtain more data relating to the cause of the appearance of sugar in the urine and other secretions, I decided to examine the lymph of the thoracic duct. I thought that over-production of sugar might coexist with a normal percentage of sugar in the blood

by virtue of the glycolytic power of the blood, which in this case might be increased. In such case one would expect to find the amount of sugar in the lymph increased after the injection of phloridzin. The experiment was conducted in the following way: The animals were allowed to fast for at least 24 hours previous to the operation. They were narcotized by means of morphine and ether. In all the operations artificial respiration was employed. In order to prevent coagulation the lymph was collected in a saturated solution of Na_2SO_4, which was then boiled with a few drops of acetic acid. The precipitate was once more boiled with a saturated solution of Na_2SO_4, again filtered, and the precipitate again washed with a hot Na_2SO_4 solution. All the filtrates and wash waters were combined, and the sugars determined by means of the following modification of Ahlin's method, which I have used for the last two years.

As in Ahlin's method, an excess of Fehling's solution was taken. After the reduction of the copper, the excess of the Fehling's solution was decanted on an asbestos filter, and the main part of the residue was left in the evaporating dish. The residue was then washed and the wash water decanted again. The precipitate of Cu_2O on the asbestos filter was also washed; and the Cu_2O in the dish and that on the filter were dissolved by means of nitric acid, and mixed together. After it had been neutralized by means of sodium carbonate, a few drops of ammonia were added to the solution, which was then titrated by means of a standard solution of KCy. This method has also been in use for the last year in the Analytical Laboratory of the School of Mines of the Columbia University of New York. The results of these experiments may be summarized as follows:

Experiment 1, *April* 17.—Dog; weight, 7 kilos; no food for 48 hours previous to the operation; 0.05 gramme of morphine two hours before the operation; ether; artificial respiration. A cannula was inserted into the thoracic duct. During the first thirty minutes 24.1 grammes of lymph were collected. Then 20 cubic centimetres of a 2 per cent solution of phloridzin in alkaline water were gradually injected into the right jugular vein, and in the next twenty minutes 36.2 grammes of lymph were collected. The urine obtained from the ureter contained sugar.

The amount of Cu reduced by the lymph before injection of phloridzin = 0.0313 gramme, corresponding to 0.078 per cent of sugar.

The amount of Cu reduced by the lymph after injection of phloridzin = 0.0686 gramme, corresponding to 0.0967 per cent of sugar.

In this case the first lymph was not collected in Na_2SO_4 at once, but was only later transferred to this solution, the greater part being then coagulated.

Experiment 2, *May* 27.—Dog; weight, 20 kilos; fasting about 24 hours previous to the operation; 0.15 gramme of morphine administered about two hours before the operation. During the first thirty-five minutes 24 grammes of lymph were collected; 28 cubic centimetres of a 2 per cent solution of phloridzin in alkaline water were then gradually injected into the right jugular vein. The lymph collected in the next twenty minutes was lost, but a third portion was collected during forty minutes equal to 43.6 grammes.

The amount of Cu reduced by the first lymph = 0.098 gramme = 0.279 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.0634 gramme = 0.074 per cent of glucose.

Experiment 3, *June* 1.—Dog; weight, 10 kilos; fasting 48 hours previous to the operation; 0.1 gramme morphine injected two hours before the operation. During the first thirty minutes after the operation 10 grammes of lymph were collected. 30 cubic centimetres of a 1 per cent solution of phloridzin were then injected into the right jugular vein. In the next forty-five minutes 22.5 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.049 gramme = 0.259 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.078 gramme = 0.177 per cent of glucose.

Experiment 4, *June* 2.—Dog; weight, 18 kilos; fasting 24 hours previous to the operation; 0.15 gramme of morphine injected one hour before the operation. During the first twenty-five minutes after the operation 29.3 grammes of lymph were collected. 0.4 gramme of phloridzin in 30 cubic centimetres of very dilute Na_2CO_3 solution was injected into the jugular vein. In the next 15 minutes 27.4 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.0625 gramme = 0.110 per cent of glucose. (Part of the Cu solution prepared for titration was lost during neutralization.)

The amount of Cu reduced by the second lymph = 0.0666 gramme = 0.125 per cent of glucose.

Experiment 5, *June* 4.—Dog; weight, 12 kilos; fasting 24 hours before the operation; 0.15 gramme of morphine injected two hours before the operation. During the first twenty minutes after the operation 24.6 grammes of lymph were collected. 0.4 gramme of phloridzin in 30 cubic centimetres of H_2O containing some Na_2CO_3 was injected into the right jugular vein.

The amount of Cu reduced by the first lymph = 0.095 gramme = 0.196 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.078 gramme = 0.175 per cent of glucose.

Experiment 6, *June* 8.—Dog; weight, 20 kilos; fasting twenty-four hours before the operation; 0.15 gramme of morphine injected two hours before the operation. During the first thirty-five minutes 3 grammes of lymph were collected. 0.3 gramme of phloridzin dissolved in 30 cubic centimetres of H_2O containing some Na_2CO_3 was then injected. During the next 45 minutes 10 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.0161 gramme = 0.300 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.0397 gramme = 0.130 per cent of glucose.

The results are here grouped in tabular form.

No.	Percentage of Glucose in the lymph before injection of phloridzin.	Percentage of Glucose in the lymph after injection of phloridzin.	Difference expressed in percentages.
1	0.078	0.096	+ 23
2	0.279	0.074	— 73
3	0.259	0.177	- 32
4	(0.110) part lost	0.125	— ?
5	0.196	0.175	- 11
6	0.300	0.130	- 57

All these experiments except No. 1 indicate a considerable decrease of sugar in the lymph after the injection of phloridzin.

In order to ascertain the relation of this decrease of glucose in the lymph to the activity of the kidneys, I thought it advisable to ligate the whole hylus renalis previous to the collection of the lymph and the injection of the phloridzin; and, in order to determine to what extent this decrease might be due to the action of phloridzin, I examined the lymph collected in the same intervals as in the other experiments, but without the injection of the glucoside. Unfortu-

nately I did not have time to perform each of these control operations more than once.

Experiment, June 12, 1896.—Dog; weight, 18 kilos; fasting twenty-four hours before the operation; 0.1 gramme morphine injected two hours before the operation. The dog was narcotized, artificial respiration was employed; both hyli renales were ligated. The operation was then directed to the thoracic duct. During the first 30 minutes 13 grammes of lymph were collected. 0.5 gramme of phloridzin in 40 cubic centimetres of H_2O containing some Na_2CO_3 was injected into the jugular vein, and in the next thirty minutes 19.5 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.063 gramme = 0.240 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.568 gramme = 0.150 per cent of glucose.

Control Experiment, June 18, 1896.—Dog; weight, 18 kilos; no phloridzin injected. The operation was performed in the usual way with anæsthetics and artificial respiration. During the first 35 minutes 16.5 grammes of lymph were collected, during the following 35 minutes 15 grammes.

The amount of Cu reduced by the first lymph = 0.0725 gramme = 0.224 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.0706 gramme = 0.240 per cent of glucose.

The foregoing experiments tend to show that the kidneys are not the only organs affected by phloridzin. This conclusion is based not only upon the result of phloridzin injection on the bile and the milk, but also upon the fact that the amount of sugar in the lymph decreases after the injection of phloridzin, even if the hyli renales are ligated previous to the injection of the glucoside.

The examination of the lymph shows a decrease of sugar after the administration of the phloridzin. If this is to be explained by the fact that the blood, being more than normally deprived of sugar by one organ, yields less to the other tissues, and, therefore, the lymph flowing from those tissues is poorer in sugar than under normal conditions, we have a strong argument in support of the elimination theory. But, on the other hand, it has been found that the urine

can eliminate during one hour after the administration of phloridzin more sugar than can be obtained from the daily amount of lymph. This fact, especially when taken in conjunction with the changes taking place in the different tissues and in the metabolism of the whole organism, offers at present some room for doubt in regard to the elimination theory. The same opinion has been expressed by Kraus.* At the same time the occurrence of spontaneous diabetes without hyperglycæmia, which has been proved by Seegen, does not justify v. Noorden in his assertion that the mechanism of phloridzin glycosuria does not resemble any form of spontaneous diabetes. Only recently Klemperer, at the meeting of the Verein für innere Medicin in Berlin (May 18, 1896), has called attention to a form of renal diabetes resembling the phloridzin glycosuria.

As to Zuntz's supposition of the existence of a certain mechanism regulating the amount of sugar in the blood through increased or decreased combustion of the sugar, I may remark that the study of phloridzin glycosuria has made virtually the same impression on myself, and I expressed in my previous paper the following opinion: "The absence of hyperglycaemia may be explained by the supposition that the organism has not lost its ability to rid itself of the superabundance of sugar on the one hand by its elimination through the kidneys, and on the other by its increased decomposition."

I wish to express my thanks to Professors J. G. Curtis and F. S. Lee for their kindness to me during my work in their laboratory.

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THE GELATIN FROM WHITE FIBROUS CONNECTIVE TISSUE.

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In a general way the chemical nature and composition of gelatin have been established for many years, but the more thorough chemical study of various forms of connective tissue, which has been carried on so successfully during the last decade, has revealed the existence of at least three distinct varieties of collagen, characterized by marked differences in chemical composition. The collagen of white fibrous tissue and of bone is generally considered as the same; it apparently yields on hydration a gelatin with about 18 per cent of nitrogen. The collagen which makes up the greater portion of the cornea, on the other hand, contains about 17 per cent of nitrogen,* while the collagen of cartilage† yields a gelatin with only 16 per cent of nitrogen. Furthermore, we have evidence of the existence of a body in reticular tissue—reticulin—the composition of which appears to lie midway between that of gelatin and of elastin. ‡ Its nitrogen-content, according to Siegfried, is 15.63 per cent. Again, the amount of sulphur in gelatin is usually placed at about 0.6 per cent,§ but the gelatin formed from corneal collagen contains only 0.3 per cent of this element.|| Lastly, Schützenberger and Burgeois consider gelatin (commercial gelatin) as practically free from sulphur, or hold at least that this element is an unessential constituent of the molecule.¶

* Mörner, Untersuchung der Proteinsubstanzen in den lichtbrechenden Medien des Auges. *Zeitschr. f. physiol. Chem.*, Bd. xviii, p. 224.

† Mörner, Studien über die Chemie des Trachealknorpels. *Jahres-Bericht d. Thier-Chemie*, 1888, p. 220.

‡ Siegfried, Ueber die chemischen Eigenschaften des reticulirten Gewebes. Habilitationsschrift, Leipzig, 1892.

§ Hammarsten's Lehrbuch d. physiolog. Chem., 1895, p. 46.

|| Mörner, *Zeitschr. f. physiol. Chem.*, Bd. xviii, p. 225.

¶ Recherches sur la constitution des matières collagènes. *Comptes rendus*, t. lxxxii, p. 262.

In all of these tissues the difficulty of isolating with perfect purity the albuminoid itself is very great. In white fibrous tissue, for example, the close juxtaposition of the several anatomical elements, as well as the similarity in the reactions of the several chemical substances present, make it an exceedingly difficult matter to insure a complete isolation of the collagen. In a general way this may be readily done, but to obtain collagen from tendons, for example, absolutely free from all traces of mucin, elastin and albuminous matter is not so easy. To be sure, the large proportion of collagen present in tendons practically insures, with reasonable care, freedom from any very large percentage of impurity; but it is generally understood among physiological chemists that the sulphur of collagen or gelatin, for example, is somewhat questionable, that at least a portion of the 0.6 per cent of this element usually accredited to gelatin may be due to admixture of the richer sulphur-containing bodies, mucin or albumin, with which it is so intimately associated in the tissue. Indeed, it seems doubtful if we have on record any analysis of collagen or gelatin extracted from white fibrous connective tissue which can be accepted as representing the chemical composition of the perfectly pure substance. Thus, a glance through the literature of the subject shows that most of our knowledge, especially bearing on the chemical composition of gelatin, is based upon a study of the commercial product, the source of which is obviously more or less uncertain. It has therefore seemed to the writer very desirable, in view of the probable existence of a number of distinct collagens and gelatins, to determine as accurately as possible the chemical composition of gelatin from white fibrous tissue, having especially in view the preparation of the substance entirely free from all traces of elastin, mucin and albumin. This seems the more important as a necessary step preliminary to comparing the reactions of gelatin with albumin; a comparison which is of primary importance in determining the exact genetic relationship of these two bodies.

In many investigations on the chemical nature and properties of gelatin, the commercial product, purified perhaps by precipitation with alcohol, etc., has been employed and with valuable results, but in the

light of present knowledge it seems quite imperative to establish with definiteness the chemical composition and reactions of gelatin from white fibrous connective tissue, and for this purpose it is necessary to have a product from a definite source and prepared in such a manner as to insure freedom from admixture, however slight, with any extraneous matters. The numerous proteid reactions obtained with ordinary gelatin are, in many cases at least, much less intense than those obtained with true proteids, a fact which has frequently led to the suggestion that gelatin owed its reactions in these cases to admixed albumin obtained from the tissue cells, etc.

When gelatin has once been prepared from the tissue-collagen, its further purification becomes exceedingly difficult. It is therefore of primary importance that the albumin, elastin and mucin be completely removed from the collagenous tissue prior to the conversion of the latter into gelatin. The removal of these substances, however, from so compact a tissue as tendon, with its predominance of collagenous fibres, is not easy, and considerable time must be given in order to accomplish this with any degree of thoroughness. The agents employed for the removal of these substances were water, half-saturated lime-water, dilute acetic acid, and alkaline pancreatic juice.

PREPARATION A.

Achilles tendons from oxen were carefully cleaned, freed from fat, etc., and then cut into very small pieces. The finely divided material (700 grammes) was then soaked in thymolized water, frequently changed, for several days to remove as much soluble matter as possible. It was next placed in 2 litres of 0.25 per cent solution of sodium carbonate; 300 cc. of a powerful pancreatic juice, prepared from Kühne's dried pancreas, added, and the mixture warmed at 40° C. for five days, thymol being added from time to time to prevent putrefaction. In an alkaline pancreatic juice of this description the pieces of tendon swell up and the individual fibres become more or less separated, so that the mass becomes soft and spongy, thus giving the solvent a good opportunity to reach every part of the tissue.

As has been frequently demonstrated,* collagenous fibres which have not been boiled with water or treated with dilute acid are insoluble in an alkaline solution of trypsin. Elastic fibres, on the other hand, undergo digestion by this treatment, with formation of soluble products, elastoses and elastin-peptone. Mucin is likewise dissolved by the alkaline fluid, while all albuminous matter is readily converted into soluble proteoses, peptone and amido-acids. This method would certainly seem to furnish an effectual means of removing all tissue-elements except the collagen; even the nucleo-proteids and nucleins of the tissue cells being dissolved by this treatment.

When the digestion was completed the residual collagen was strained off and washed thoroughly in large volumes of water until the pieces of tissue were without action on litmus paper and the fluid gave no reaction for dissolved proteids. It is to be noted, however, that when such apparently neutral tissue is boiled with water, the fluid gradually acquires an alkaline reaction, thus indicating that the last traces of alkali cannot be removed by water alone. The collagen was next boiled with several litres of water, each portion of water added being kept in contact with the collagen for a few hours only, in order to avoid the formation of any gelatoses by the too prolonged heating of the newly formed gelatin. For the same reason water was frequently added to prevent the boiling point of the solution being raised by the increasing concentration. When the first extract was poured off, the residual collagen was boiled with a fresh quantity of distilled water, this operation being repeated until the larger part of the collagen was transformed into gelatin. The united fluids were then evaporated on the water-bath until quite concentrated, forming when cold a hard, firm jelly. The gelatin was next precipitated by alcohol, this being accomplished by pouring the warm jelly, with stirring, into a large volume of 95 per cent alcohol, by which it was thrown down as a soft stringy mass, gradually hardening by longer contact with the alcohol. The precipitate was then extracted for some days with absolute alcohol, and then with ether until it became hard and brittle. It was next

* See Ewald, Zur Histologie und Chemie der elastischen Fasern und des Bindegewebes. *Zeitschr. f. Biol.*, Bd. xxvi, p. 1.

ground up in a mortar, and the powder extracted continuously for two days with ether, for the complete removal of all fat or other ether-soluble matter. After this treatment the gelatin was dissolved in hot water, the fluid filtered through paper with the aid of a hot-water funnel, the filtrate concentrated somewhat, and again precipitated by a large volume of alcohol. The precipitate was dehydrated by repeated treatment with absolute alcohol, again pulverized and re-extracted with ether, after which it was considered ready for analysis.

For analysis, a sufficient amount of the substance was dried at 110° C. until of constant weight. Carbon and hydrogen were determined by combustion in an open tube with oxygen gas, the products of combustion passing over a layer of cupric oxide, a layer of lead chromate and a roll of reduced copper. Nitrogen was determined by the Kjeldahl method, sulphur by fusion with sodium hydroxide and potassium nitrate in a silver crucible and precipitation as barium sulphate after the method recommended by Hammarsten.*

Following are the results obtained:

PREPARATION A.

I. 0.5359 gramme substance gave 0.9818 gramme $CO_2 = 49.96$ per cent C, and 0.3203 gramme $H_2O = 6.64$ per cent H.
II. 0.3622 gramme substance gave 0.6615 gramme $CO_2 = 49.87$ per cent C, and 0.2145 gramme $H_2O = 6.57$ per cent H.
III. 0.2789 gramme substance gave 0.5118 gramme $CO_2 = 50.04$ per cent C, and 0.1650 gramme $H_2O = 6.57$ per cent H.
IV. 0.2006 gramme substance gave 0.03565 gramme $N = 17.77$ per cent N.
V. 0.1934 " " " 0.03435 " $N = 17.76$ " N.
VI. 0.9458 " " " 0.0214 " $BaSO_4 = 0.509$ per cent S.
VII. 0.8236 " " " 0.0202 " $BaSO_4 = 0.336$ " S.
VIII. 0.6468 " " " 0.0021 " ash $= 0.324$ " ash.

COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	VI.	VII.	Average.
Carbon,	50.12	50.03	50.21	.....	.....	.....	.....	50.12
Hydrogen,	6.66	6.59	6.59	.....	.....	.....	.....	6.61
Nitrogen,	.....	.....	.....	17.82	17.86	.....	.....	17.84
Sulphur,	.....	.....	.....	.....	.....	0.310	0.336	0.322
Oxygen,	.....	.....	.....	.....	.....	.....	.....	25.10

* *Zeitschr. f. physiol. Chemie*, Bd. ix, p. 289.

PREPARATION B.

This preparation was made from calves' tendons, Achilles tendons, the method pursued being exactly similar to that made use of in preparation A.

The composition of the gelatin, dried at 110° C. until of constant weight, was as follows:

I. 0.2773 gramme substance gave 0.5088 gramme CO_2 = 50.03 per cent C, and 0.1597 gramme H_2O = 6.39 per cent H.
II. 0.2711 gramme substance gave 0.4957 gramme CO_2 = 49.86 per cent C, and 0.1614 gramme H_2O = 6.61 per cent H.
III. 0.6762 gramme substance gave 1.2323 gramme CO_2 = 49.69 per cent C, and 0.3934 gramme H_2O = 6.46 per cent H.
IV. 0.4643 gramme substance gave 0.8476 gramme CO_2 = 49.73 per cent C, and 0.2734 gramme H_2O = 6.54 per cent H.
V. 0.2469 gramme substance gave 0.04409 gramme N = 17.85 per cent N.
VI. 0.2105 " " " 0.03754 " N = 17.79 " N.
VII. 0.6945 " " " 0.0115 " $BaSO_4$ = 0.226 per cent S.
VIII. 0.7304 " " " 0.0127 " $BaSO_4$ = 0.238 " S.
IX. 1.0424 " " " 0.0180 " $BaSO_4$ = 0.236 " S.
X. 0.3090 " " " 0.0010 " ash = 0.323 " ash.
XI. 0.4839 " " " 0.0020 " ash = 0.413 " ash.

COMPOSITION OF THE ASH-FREE SUBSTANCE.

(Average ash = 0.368 per cent.)

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	Average.
Carbon,	50.21	50.04	49.87	49.91	.....	.....	.....	.....	.....	50.01
Hydrogen,	6.41	6.63	6.48	6.56	.....	.....	.....	.....	.....	6.52
Nitrogen,	.....	.....	.....	.....	17.91	17.85	.....	.....	.....	17.88
Sulphur,	.....	.....	.....	.....	.....	.....	0.226	0.238	0.236	0.233
Oxygen,	.....	.....	.....	.....	.....	.....	.....	.....	.....	25.35

PREPARATION C.

Here the method of preparing the collagen was somewhat different from that followed in the two preceding cases. Ox tendons, carefully cleaned and cut into thin transverse sections with a razor (800 grammes), were extracted thoroughly with water, then soaked in half-saturated lime-water for three days, for the removal of the mucin. The residual tissue was washed with water and again extracted with lime-water as before. After this treatment the tissue was washed repeatedly for five days with water slightly acidified with acetic acid, care being taken to avoid adding enough acid to cause swelling of the

fibrous tissue. It was then digested with alkaline pancreatic juice at 40° C., 2.3 litres of an active solution of trypsin containing 0.25 per cent of sodium carbonate being employed. After five days' treatment of this kind the residual collagen was strained off, washed repeatedly with cold water until entirely free from alkaline reaction, after which it was converted into gelatin by the method already described, and further purified as detailed under preparation A.

Analysis of the product, dried at 110° C. until of constant weight, gave the following results:

I. 0.3504 gramme substance gave 0.6434 gramme CO_2 = 50.07 per cent C. and 0.2086 gramme H_2O = 6.61 per cent H.
II. 0.5127 gramme substance gave 0.9390 gramme CO_2 = 49.94 per cent C.

III.	0.3711	"	"	"	0.06601	"	$N = 17.78$ " N.
IV.	0.1825	"	"	"	0.03245	"	$N = 17.78$ " N.
V.	1.1582	"	"	"	0.0206	"	$BaSO_4 = 0.243$ per cent S.
VI.	1.3220	"	"	"	0.0172	"	$BaSO_4 = 0.177$ " S.
VII.	0.6631	"	"	"	0.0107	"	$BaSO_4 = 0.221$ " S.
VIII.	0.5881	"	"	"	0.0017	"	ash = 0.289 " ash.
XI.	0.2548	"	"	"	0.0009	"	ash = 0.353 " ash.

COMPOSITION OF THE ASH-FREE SUBSTANCE.

(Average ash = 0.321 per cent.)

	I.	II.	III.	IV.	V.	VI.	VII.	Average.
Carbon,	50.23	50.10	.....	.....	.....	.....	.....	50.16
Hydrogen,	6.63	.....	.....	.....	.....	.....	.....	6.63
Nitrogen,	.....	.....	17.83	17.83	.....	.....	.....	17.83
Sulphur,	.....	.....	.....	.....	0.243	0.177	0.221	0.213
Oxygen,	.....	.....	.....	.....	.....	.....	.....	25.14

PREPARATION D.

In this preparation the method of procedure was not quite so elaborate as in the three preceding cases. The tendons from oxen were cleaned and cut into thin transverse sections, soaked for some time in water, then extracted for five days with half-saturated lime-water, occasionally changed. The residual tissue was next washed for ten days with water slightly acidified with acetic acid, lastly with water alone. It was then boiled with water for about half an hour, the resultant solution being rejected, after which the collagen was transformed into gelatin as in the preceding cases, except that the boiling

was not continued for so long a period, in order to avoid any hydration of the elastin that might be present. The gelatin was then purified as in the preceding cases.

Analysis of the dried product gave the following results:

I. 0.3019 gramme substance gave 0.5528 gramme $CO_2 = 49.93$ per cent C, and 0.1754 gramme $H_2O = 6.45$ per cent H.

II. 0.3599 gramme substance gave 0.6613 gramme $CO_2 = 50.10$ per cent C, and 0.2107 gramme $H_2O = 6.53$ per cent H.

III. 0.2181 gramme substance gave 0.03865 gramme $N = 17.72$ per cent N.

IV. 0.1649 " " " 0.02902 " $N = 17.60$ " N.

V. 0.6478 " " " 0.0111 " $BaSO_4 = 0.234$ per cent S.

VI. 1.2868 " " " 0.0241 " $BaSO_4 = 0.256$ " S.

VII. 1.2868 " " " 0.0270 " $BaSO_4 = 0.287$ " S.

VIII. 0.3640 " " " 0.0010 " ash $= 0.274$ " ash.

IX. 0.4274 " " " 0.0013 " ash $= 0.304$ " ash.

Composition of the Ash-free Substance.

(Average ash = 0.289 per cent.)

	I.	II.	III.	IV	V.	VI.	VII.	Average.
Carbon,	50.07	50.24	.....	.....	.....	.....	.....	50.15
Hydrogen,	6.46	6.54	.....	.....	.....	.....	.....	6.50
Nitrogen,	.....	.....	17.77	17.65	.....	.....	.....	17.71
Sulphur,	.....	.....	.....	.....	0.234	0.256	0.287	0.259
Oxygen,	.....	.....	.....	.....	.....	.....	.....	25.38

Average Composition of the Different Preparations, Ash-free.

	A.	B.	C.	D.	Average.
Carbon,	50.12	50.01	50.16	50.15	50.11
Hydrogen,	6.61	6.52	6.63	6.50	6.56
Nitrogen,	17.84	17.88	17.83	17.71	17.81
Sulphur,	0.322	0.233	0.213	0.259	0.256
Oxygen,	25.10	25.35	25.14	25.38	25.24
Ash,	0.324	0.368	0.321	0.289	0.325

The results of these analyses agree so closely that it is safe to conclude that the several preparations have practically the same composition. Especially noticeable is the low content of sulphur and the small content of ash, two features which may be taken as indicative of the great purity of the products. Undoubtedly the small percentage of sulphur, as contrasted with those in the older analyses, is due to the more complete removal of mucin in these preparations, for, as has been recently found, the sulphur-content of mucin from tendons

is quite high, 2.32 per cent.* It is also to be noted that the present analyses show the percentage of sulphur in this form of gelatin to be even lower than the amount found by Mörner in the gelatin obtained from corneal cartilage, 0.3 per cent. Further, the above samples of gelatin give no reaction for mercaptan-sulphur with plumbic acetate and potassium hydroxide.

In preparation C, phosphorus was determined by fusion with sodium hydroxide and potassium nitrate, etc. The result showed the presence of 0.025 per cent of phosphorus. The ash, however, contained calcium phosphate. Calculating all the phosphorus found to tribasic calcium phosphate would account for less than half the ash. The ash also contained calcium sulphate. Taking the average content of ash and calculating half of it as calcium sulphate would require 0.038 per cent of sulphur, an amount too small to materially modify the percentage of sulphur found in the substance.

Among the earlier analyses of gelatin, or rather of collagen, from white fibrous connective tissue are those of Scherer.† His process of preparation consisted in macerating the tendons in water containing saltpetre, washing repeatedly with water, and finally extracting with alcohol and ether. The average composition of the product was C 50.51, H 7.16, N 18.37, S and O 24.03.

Hofmeister‡ prepared a body which he considered identical with natural collagen, by heating commercial gelatin (purified by precipitation with alcohol and containing 0.61 per cent of ash) to 130° C. until constant weight was attained. This substance he believed to differ from the gelatin by only one molecule of water. Its composition was C 50.75, H 6.47, N 17.86, S and O 24.92. It contained 0.62 per cent of ash. Sulphur was not determined.

Gorup-Besanez§ gives the following analysis of tendons and of the gelatin prepared from them:

* Chittenden and Gies, The Mucin of White Fibrous Connective Tissue. This Journal, vol. I, p. 186.

† *Annal. d. Chem. u. Pharm.*, Bd. xl, p. 46.

‡ Ueber die chemische Structur des Collagens. *Zeitschr. f. physiol. Chem.*, Band ii, p. 299.

§ Lehrbuch d. physiol. Chem., 1878, p. 142.

	Tendons.	Gelatin.
Carbon,	50.9	50.2
Hydrogen,	7.2	6.7
Nitrogen,	18.3	17.9
Sulphur, Oxygen,	23.5	25.0

The content of sulphur in gelatin he gave as 0.56 per cent.

Chittenden and Solley,* by an analysis of commercial gelatin, purified by precipitation with alcohol, etc., obtained the following results: C 49.38, H 6.81, N 17.97, S 0.71, O 25.13. The ash content was 1.26 per cent.

Hammersten† obtained 0.665–0.747 per cent of sulphur in commercial gelatin containing 1.74 per cent of ash.

Weiske‡ prepared gelatin or gelatoses from bone by extracting with dilute hydrochloric acid and then heating the residual tissue with water at 130° C. in a Papin's digester. This product contained only 0.30 per cent of ash. The disadvantage of extracting tissue of this kind with hydrochloric acid, although unavoidable in the study of bone-collagen, is apparent from his own statement that after washing the softened tissue in water for four weeks it still contained chlorine. From commercial gelatin, according to O. Nasse,§ the inorganic salts may be removed by long-continued soaking in water. In this way he was able to reduce the ash to 0.6 per cent. The soaking of gelatin in dilute acid, however, results in the formation of a salt-like compound, from which the acid cannot be withdrawn by washing with water. By adding a little ammonia to the water this may be accomplished.

From this short résumé it is evident that pure gelatin from white fibrous connective tissue is not widely different in composition from commercial gelatin, so far as the content of carbon, hydrogen and nitrogen is concerned. In the content of sulphur, however, there is a wide difference, pure gelatin from tendons containing only 0.25 per cent of this element. Especially important in this connection is the fact, now brought out, that the sulphur-content of gelatin from white

* The Primary Cleavage Products formed in the Digestion of Gelatin. *Journal of Physiol.*, vol. xii, p. 23.

† *Zeitschr. f. physiol. Chem.*, Band ix, p. 305.

‡ Zur Chemie des Glutins. Ibid., Bd. vii, p. 460.

§ Ueber die Chemie des Glutins. *Jahres-Bericht d. Thier-Chemie*, 1889, p. 29.

fibrous connective tissue is very closely akin to that of the related albuminoid elastin,* the latter, according to the latest researches, containing 0.3 per cent. of this element. Elastin, however, differs widely from gelatin in its content of carbon and nitrogen. Further, the fancied difference in the content of sulphur between corneal gelatin and tendon gelatin also falls away, although these bodies are plainly unlike in their content of nitrogen.

REACTIONS OF GELATIN.

Although the four samples of gelatin described were studied with great care, no difference in chemical reactions was observed. They all showed a very faint alkaline reaction and were rather noticeably soluble in cold water. In trying precipitations with various reagents, a 2 per cent aqueous solution of gelatin was made use of, and at room temperature this was fluid enough for the purpose, but if warmed and allowed to cool undisturbed the solution showed some tendency to gelatinize. Halliburton† states that the power of gelatinizing is possessed by solutions from 1 per cent up, but diminishes with every successive solution. This somewhat weak power of gelatination is not to be ascribed to extensive conversion into gelatoses, but is more probably connected with the greater purity of the products. Thus O. Nasse‡ observed that the more nearly free from ash his gelatin was, the less the tendency to gelatinize, diminution in the percentage of ash leading to loss of the power of gelatination. This being the case, freedom from inorganic constituents might naturally be expected to result in greater solubility in cold water. Hammarsten§ mentions the well-known fact that gelatin is more easily precipitated by alcohol in the presence of neutral salts, and he further mentions that "it is precipitated by tannic acid in the presence of salt." Salts unquestionably do exert a very marked influence in rendering precipitation

* Chittenden and Hart, Elastin and Elastose Bodies. *Studies in Physiol. Chem.*, Yale Univer., vol. iii, p. 19.

Schwarz, Untersuchungen über die chemische Beschaffenheit der elastischen Substanz der Aorta. *Zeitschr. f. physiol. Chem.*, Bd. xviii, p. 487.

† Text-book of Chem. Physiol. and Pathol., p. 471.

‡ *Jahres-Bericht d. Thier-Chemie*, 1889, p. 31.

§ Lehrbuch d. physiol. Chem., 1895, p. 47.

of gelatin more complete, from which it might be argued that their absence from or diminution in gelatin would enhance its solubility in water and decrease its power of gelatinizing. On the other hand, it is of course possible that the long-continued process of preparation may have resulted in the formation of some gelatose, although great care was exercised to avoid such an occurrence, the heating with water in the conversion of collagen into gelatin having been of comparatively short duration.

In the precipitation of pure gelatin with most reagents there is noticeable a tendency toward incompleteness of separation and a dependence of the reaction upon the attendant conditions, especially the concentration of the fluid. Upon this fact, no doubt, many of the conflicting statements in regard to the reactions of gelatin depend, although some of the differences noted by various writers are due to the lack of purity of the gelatin employed, and possibly also to the source from which it was derived. Further, the fact that with certain reagents only slight precipitates are obtained has led to the belief that these reactions are due to the presence of albumin and not to gelatin itself, whereas the correct explanation in many cases is to be found in the solubility of the precipitate, in the excess of the reagent, for example, which may even prevent its appearance altogether if the conditions are not favorable.

Pure gelatin gives the biuret or Piotrowski's reaction strongly, the shade of color being a violet-purple. Millon's and the xanthoproteic reactions are obtained with great distinctness, the former being especially strong, although in both cases the reaction is by no means as marked as that obtained with corresponding amounts of albumin or globulin. The reactions are altogether too pronounced to be credited to traces of contained albumin, as has frequently been done in the past. Far more plausible is it to assume that the radical or radicals (perhaps an oxybenzene group) to which these reactions are due, and which are so abundant in the proteid molecule, are contained only in small proportion in the gelatin molecule.

With Adamkiewicz's reaction, and also with Liebermann's reaction, pure gelatin yields negative results.

With acetic acid and potassium ferrocyanide a slight precipitation is obtained, if the ferrocyanide solution is added carefully. A very slight excess of the latter redissolves the precipitate.

With picric acid, trichloracetic acid, tannic acid, chloroplatinic acid, phosphomolybdic acid and phosphotungstic acid, pure gelatin is more or less completely precipitated. With picric acid the precipitate first formed redissolves readily, becoming permanent on addition of more of the reagent. It is somewhat soluble in excess of the precipitant. With trichloracetic acid the precipitation is far from complete.

The following reagents do not precipitate pure gelatin: plumbic acetate, basic lead acetate, cupric sulphate, ferric chloride, silver nitrate, and mercuric chloride.

Mercuric chloride, however, causes a precipitate if hydrochloric acid is added. As solutions of mercuric chloride sometimes contain a little free acid, a turbidity may be obtained with the mercuric chloride alone. The precipitate formed on adding acid is very soluble in excess of acid, especially on warming the fluid, reappearing as the solution cools. Sodium chloride appears to assist in the precipitation if added in considerable quantity (in 20 per cent solution), simply by making a medium in which the precipitate is less soluble, but it does not play any essential part in the reaction. Acetic acid will not take the place of hydrochloric acid in this reaction, neither will sodium chloride induce precipitation of gelatin by mercuric chloride in the absence of acid.

Gelatin is precipitated from aqueous solutions by saturation of the fluid with ammonium sulphate, with sodium sulphate and with magnesium sulphate.

Saturation with sodium chloride causes only a slight precipitate, but if acetic acid is added to the salt-saturated fluid a heavy flocculent precipitate results. Hydrochloric acid will take the place of acetic acid in this reaction, but the precipitate is more soluble in excess of the acid.

Saturation with ammonium chloride fails to produce any precipitate until the fluid is acidified with acetic acid, when the gelatin, in part at least, is thrown down.